In-silico screening of potential inhibitors of HMGCoA reductase and Lanosterol synthase, key enzymes in Cholesterol biosynthesis pathway

A thesis submitted in partial fulfilment of the requirements for the degree of

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In

Biotechnology

By

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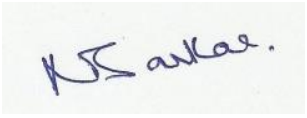
CERTIFICATE

This is to certify that the project thesis report entitled "***In-silico screening of potential inhibitors of HMGCoA reductase and Lanosterol synthase, key enzymes in Cholesterol biosynthesis pathway***" submitted **by N SAI VENKATA SARATH CHANDRA (110BT0626)** in the partial fulfilment of the requirement for the degree of the B.Tech in Biotechnology in Department of Biotechnology and Medical Engineering, National Institute of Technology, Rourkela is a record of an authentic bonafide research work carried out by him under my supervision and guidance.

To the best of my knowledge, the matter embodied in the project thesis report has not been submitted to any other Institute/University for any Degree or Diploma.



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Last but not the least, I would like to thank my Parents and God for their constant encouragement and day to day support in all aspects.

Submitted by

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ABSTRACT

Hyperlipidemia is elevated level of lipids or lipoproteins in our body. Cholesterol is a major lipid particle circulating in our body. Cholesterol synthesis takes place in liver and this biosynthesis pathway is called Mevalonate pathway. Cholesterol is the end product of this Mevalonate pathway. Several enzymes play a role in the Cholesterol biosynthesis. Enzymes like HMGCoA synthase, HMGCoA reductase, Farnesyl PP synthase, Lanosterol synthase, Squalene synthase. This cholesterol is mainly responsible for several health effects especially Coronary heart diseases, atherosclerosis etc. So now in order to prevent the cholesterol synthesis in our body, we need to find potent inhibitors against the above enzymes and there after modify them as drugs. In this study, two enzymes HMGCoA reductase and Lanosterol synthase were targeted. For our docking study, we used Arguslab, Mgl tools, Autodock vina softwares for obtaining binding energies of protein and ligands. We search for already available drugs acting against HMGCoA reductase called Statins. We obtained binding energy results with NADH, PRAVASTATIN, LOVASTATIN, CERIVASTATIN, SIMVASTATIN, FLUVASTATIN, BEZAFIBRATE and note them as POSITIVE CONTROL 1. Then we dock HMGCoA reductase with natural molecules and compare them with positive control 1. In the next step, we targeted Lanosterol Synthase with available Quinuclidine inhibitors and results obtained was noted as POSITIVE CONTROL 2. Now we cross docked Lanosterol synthase with previous natural ligands and compared them with Positive control 2. We later found the toxicity and druglikeness of all inhibitors used against both the targets. We found two natural molecules APIGENIN and NARINGENIN which showed best binding energy results against both the targets. Both Apigenin and Naringenin act as suitable inhibitors against HMGCoA reductase and Lanosterol synthase inhibiting at two places along the Mevalonate pathway**.**

KEY WORDS -

Hyperlipidemia, cholesterol, HMGCoA reductase, Lanosterol synthase, Statins, Apigenin, Naringenin

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CHAPTER 1

INTRODUCTION

1. INTRODUCTION

Hyperlipidemia or hypercholesterolemia is an abnormal elevation in the level of lipids or lipoproteins in our body. Usually level of lipid rises in the blood. Cholesterol is a lipid circulating in our blood. Hypercholesterolomia is a specific case of hyperlipidemia where the level of cholesterol increases in the blood. Cholesterol is insoluble in water, it is transported in the blood plasma with other lipoproteins.Different classifications of lipoproteins include-Very Low Density Lipoprotein(VLDL), Low Density Lipoprotein(LDL), High Density Lipoproteins(HDL), Intermediate Density Lipoprotein(IDL). Cholesterol is carried by all lipoproteins and usually LDL cholesterol is risky known as Bad Cholesterol. It is known to cause Coronary heart disease and atherosclerosis. Most cholesterol in our body is produced due to internal synthesis in liver. Other sources include dietary sources, genetic influence. Cholesterol has many functions in our body like absorption of fats and hormones like testosterone, estrogen, progesterone, cortisol etc. Cholesterol helps for production of Vitamin-D in the presence of sun light. It is essential for providing structural support to cell membranes, serves as antioxidant, and helps in conduction of nerve impulses.

An important pathway takes place in liver called Cholesterol synthesis pathway or Mevalonate pathway. HMGCR or HMG-CoA reductase is an important enzyme which controls the rate of the pathway. Cholesterol will be the final product of this pathway. Other important enzymes involved in this pathway include Lanosterol synthase, Farnesyl diphosphate synthase, HMG-CoA synthase. Detailed analysis of pathway includes one molecule of Acetyl-CoA combining with one molecule of Acetoacetyl-CoA forming HMG-CoA . HMG-CoA is reduced to Mevalonic acid/Mevalonate by an enzyme called HMG-CoA reductase. Mevalonate/Mevalonic acid is now converted to Isopentyl pyrophosphate. Isopentyl pyrophosphate gets converted to Farnesyl pyrophosphate in the presence of Farnesyl-PP-Synthase. Two molecules of Farnesyl pyrophosphate condense to forming Squalene by action involving Squalene synthase. Squalene cyclists forming Lanosterol. Lanosterol gets converted to Cholesterol finally in the presence of Lanosterol synthase.

Now we choose to important enzymes as targets for our study. They are HMG-CoA reductase and Lanosterol synthase. HMG-CoA reductase is a major target for cholesterol lowering drugs. We study about a class of already available inhibitors to HMG-CoA reductase in the market called as Statins. Statins are found to prevent cardiovascular diseases and other cholesterol diseases. By inhibiting HMG-CoA reductase, Statins block the synthesis pathway in liver. Now by reduction of cholesterol production in the liver, level of cholesterol in blood falls. Examples of Statins are Fluvastatin, Lovastatin, Simvastatin, Mevastatin, Pravastatin, Atorvastatin, Rosuvastatin, Cerivastatin. Now we perform docking study retrieving PDB ID of our target, predicting the active sites of our target, perform docking by using docking tools. We choose protein model with PDB ID : 1DQ9 for HMG-CoA reductase. Predict active site catalytic portions using CASTP and obtain GLU559, LYS691, ASP767 and HIS866 as aminoacid catalytic portions. We perform docking using Autodock Vina, ArgusLab and obtain binding energies with different inhibitors both predicted and already available inhibitors. Potential molecules that are reported from this study can be assayed for development into new drugs. Similarly we choose PDB ID: 1W6J for Lanosterol synthase, predict the active sites of Lanosterol found to be HIS232, ASP455 and perform similar docking study as done in the case of HMG-CoA reductase. We identify a class of Quinuclidine inhibitors for Lanosterol synthase. Other classes are identified as Azole inhibitors and Novel-4-piperidino pyridine inhibitors. We predict the toxicity and druglikeliness of all our inhibitors.

CHAPTER 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 HYPERLIPIDEMIA/LIPIDS/LIPOPROTEIN/CHOLESTEROL

Hyperlipidemia involves high level of lipids or lipoproteins in our blood. It is a form of dyslipidemia. Cholesterol circulating in our blood when rises to abnormal level forms Hypercholesterolemia [3]. Lipoprotein is a biochemical assembly of both lipid and protein. They allow mobilization of fats and water through cell membranes. Lipids include fats, sterols, waxes, glycerides, vitamins etc. Hyperlipidemia is bound to cause coronary heart diseases, ischemic heart diseases, atherosclerotic heart diseases. Arteries get blocked due to cholesterol deposition, leading to narrowing, blocking reducing blood flow in heart. Cholesterol is a lipid particle which circulates in our body. Cholesterol acts as a crucial substance and performs some vital functions in our body.

Lipoproteins are classified into -

1. Very Low Density Lipoproteins (VLDL)
2. Low Density Lipoprotein (LDL)
3. Intermediate Density Lipoprotein (IDL)
4. High Density Lipoprotein (HDL)
5. Triglycerides

2.2 CAUSES OF HYPERLIPIDEMIA

There are many factors for the elevation of lipid level in our body. It is found that food habits, daily living conditions, stress, obesity etc may induce the level of lipid in our body. Some of the causes are found to be -

- Obesity
- Stress
- Food habits (Eating food containing saturated fats, trans fats etc)
- Age factor
- Smoking
- Genetic factor (Heredity)
- Metabolic syndrome
- Hypothyroidism

- Main cause of lipid elevation is internal biosynthesis of cholesterol which takes place in the liver.

Internal biosynthesis mechanism is the main cause for high lipid level elevation in our body. Many factors exacerbate the rate of production of lipids in our body.

2.3 DETAILED DESCRIPTION OF BIOSYNTHESIS PATHWAY OF CHOLESTEROL IN LIVER

One molecule of Acetyl COA and one molecule of Acetoacetyl COA combine to form HMGCoA. HMGCoA is reduced to Mevalonate by an enzyme HMGCoA reductase. Mevalonate is now converted to Isopentylpyrophosphate. Isopentylpyrophosphate is converted to Farnesylpyrophosphate. Two molecules of Farnesylpyrophosphate condense to form Squalene by action of Squalene by Squalenesynthase. Squalenesynthase cyclizes to form Lanosterol in the presence of Lanosterolsynthase. Finally Lanosterol gets converted Cholesterol.

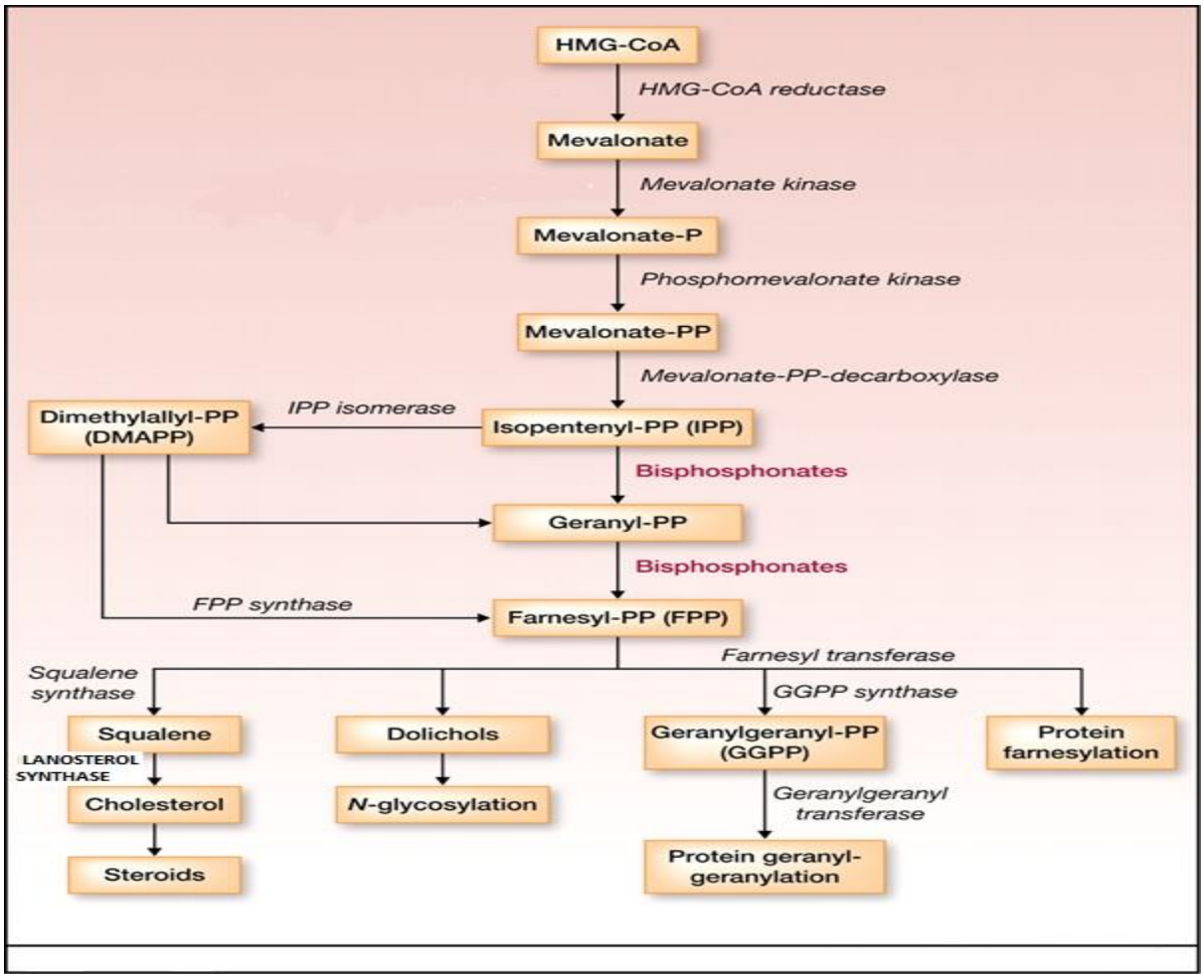


Figure 1: Shows mevalonate pathway i.e biosynthesis of Cholesterol in Liver (Reference: AACR publications)

2.4 ROLE OF DIFFERENT ENZYMES IN CHOLESTEROL SYNTHESIS PATHWAY

1. Enzyme HMGCoA synthase binds to substrate Acetoacetyl CoA giving rise to HMGCoA.
2. Enzyme HMGCoA reductase binds to substrate HMGCoA giving rise to Mevalonic acid.
3. Enzyme Mevalonate kinase binds to substrate Mevalonic acid giving rise to Mevalonate-5-phosphate
4. Enzyme Phosphomevalonate kinase binds to Mevalonate-5-phosphate giving rise to Mevalonate-5-pyrophosphate.
5. Enzyme Mevalonate-5-pyrophosphate decarboxylase binds to Mevalonate-5-pyrophosphate giving rise to Isopentyl-5-pyrophosphate.
6. Enzyme Farnesyl PP synthase converts Isopentyl-5-pyrophosphate to Farnesyl PP.
7. Later Lanosterol synthase converts Squalene to Lanosterol.
8. In the last step, Squalene lanosterol gets converted to Cholesterol

2.5 AVAILABLE DRUGS AGAINST HMGCoA REDUCTASE: THE STATINS [10]

There are a class of already available drugs called Statins used to lower cholesterol levels in our body by inhibiting HMGCoA reductase. Statins [14] are been found to prevent cardiovascular diseases and other diseases caused by elevated level of Cholesterol. By inhibiting HMGCoA reductase, statins block the pathway for synthesis of cholesterol in the liver. By reducing or checking Cholesterol production in the liver, cholesterol levels in the blood fall. Statins act by competitively inhibiting HMGCoA reductase [12]. Statins are almost similar to HMGCoA on molecular level and thus take place of HMGCoA. This reduces the rate by which mevalonate is produced in liver thereby blocking the pathway. Cholesterol synthesis occurs mostly in the nights, so statins usually having short half-lives are usually taken at night to maximize their effect. Statins are HMGCoA Reductase inhibitors [17] with an inhibition constant value in the range of nanomolar level and effectively lower amount of cholesterol present in blood serum. Statins are extensively prescribed and used for the treatment of hypercholesterolemia/hyperlipidemia. Statins usually occupy an amino acid residue portion of the binding site of HMGCoA, thus blocking the access of substrate HMGCoA to the active site catalytic portion of enzyme HMGCoA reductase. Near the carboxyl terminus of HMGCoA reductase, we find many catalytically pertinent amino acid residues which get disordered in the complex formation of HMGCoA reductase-statin complexes. Flexibility of these residues plays a major role in complex formation as they would hinder the statin binding sterically.

2.6 AVAILABLE INHIBITORS AGAINST LANOSTEROL SYNTHASE

Lanosterol synthase is an Oxidsqualene cyclase enzyme which converts 2,3 Oxidosqualene to a protosterol cation which finally gets converted into Lanosterol synthase [7]. Lanosterol is a four ringed intermediate in the formation of cholesterol. Today, interest has grown to use inhibitors of Lanosterol synthase as drugs to lower the level of Cholesterol in the blood and also to treat wide variety of diseases caused by increased level of Cholesterol like Atherosclerosis, Coronary heart diseases etc. More interest is grown when Statins showed many side effects.

A class of Quinuclidine inhibitors [6], Piperidino-pyridine inhibitors, AZOLE inhibitors are found to inhibit the enzyme substrate binding and prevent the pathway from proceeding further.

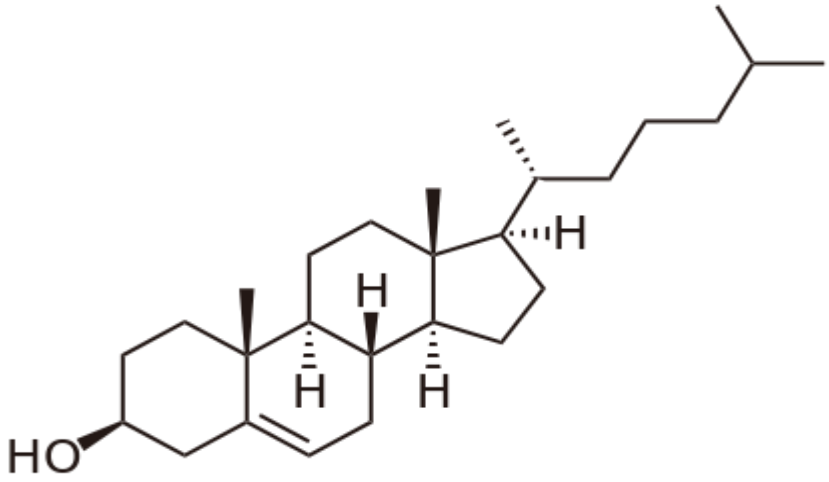


Figure 2: Shows chemical structure of Cholesterol with molecular formula $C_{27}H_{46}O$

CHAPTER 3

OBJECTIVES

3. OBJECTIVES

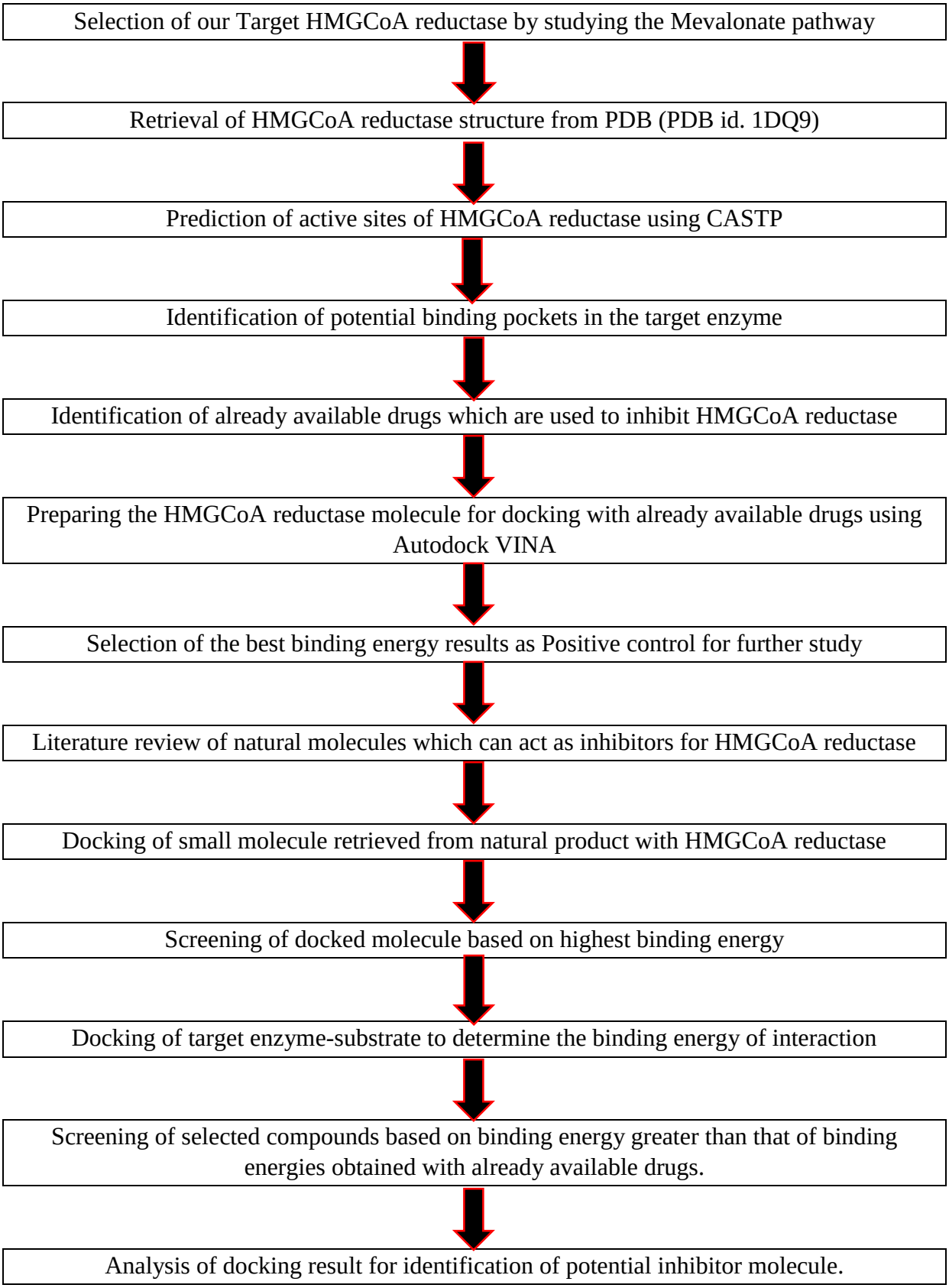
- Selection of required targets in the pathway.
- Virtual screening of target with already available inhibitors.
- Prediction of active sites of our target.
- Identifying a range of binding energies obtained by docking with already available inhibitors.
- Screening of selected natural compounds based on their binding energies near the active site of the enzyme.
- Determining binding energies obtained by docking with natural compounds.
- Screening of selected compounds based on binding energy greater than or in the range of binding energies obtained with available inhibitors.
- Analysing the docking result for identification of potential inhibitor molecule.
- Prediction of toxicity of all inhibitors used against our targets.
- Calculating Druglikeness properties like LogP, Molar refractivity of all inhibitors.

CHAPTER 4

PLAN OF WORK

4. PLAN OF WORK

PART I



PART II

Identification of another Target Lanosterol Synthase in the same pathway.

↓

Retrieval of PDBID of Lanosterol Synthase from PDB (PDBID 1W6J)

↓

Finding out inhibitors of Lanosterol Synthase

↓

Search for related compounds in PUBCHEM

↓

Prediction of active sites of Lanosterol synthase using CASTP

↓

Docking of these inhibitors with Lanosterol synthase

↓

Choosing the Binding energy values obtained with these as Positive control in this case.

PART III

Cross docking of natural molecules used for our 1st target HMGCoA reductase with our 2nd target Lanosterol Synthase.

↓

Comparing them with positive control obtained in PART2

↓

Identification of best Binding energies of natural ligands docked with Lanosterol synthase.

↓

Prediction of Toxicity of the inhibitors.

↓

Prediction of Drug likeness of these inhibitors

↓

Molecular visualization of all inhibitors in PYMOL

CHAPTER 5

MATERIALS AND METHODS

5. MATERIALS AND METHODS

5.1 MATERIALS:

5.1.1 REQUIREMENT OF FILES:

- ✓ PDB file of HMGCoA reductase (PDB ID 1DQ9) and Lanosterol synthase (1W6J)
- ✓ Converting .pdb to .pdbqt using MGL tools
- ✓ Geometry optimization using Arguslab
- ✓ mol file of ligand to find the toxicity

5.1.2 REQUIREMENT OF SOFTWARES:

- ✓ Autodock Vina
- ✓ Arguslab
- ✓ MGL tools
- ✓ Ibabel
- ✓ PYMOL

5.1.3 REQUIREMENT OF ONLINE SERVERS:

- ✓ http://www.rcsb.org/
- ✓ bioserver-3.bioacademy.gr/Bioserver/ChemBioServer/Toxic.php
- ✓ http://www.drugbank.ca/
- ✓ http://www.uniprot.org/
- ✓ http://www.ncbi.nlm.nih.gov
- ✓ www.chemicalize.org
- ✓ http://sts.bioengr.uic.edu/castp/

5.2 METHODS:

In this Insilco based screening, we have selected HMGCoA reductase (PDB ID 1DQ9) and Lanosterol Synthase (PDB ID 1W6J) both of which play a critical role in the cholesterol biosynthesis in liver

5.2.1 PREPARATION AND SELECTION OF TARGET MOLECULES OF HMGCOA REDUCTASE, LANOSTEROL SYNTHASE:

HMGCoA reductase (PDB ID 1DQ9) and Lanosterol synthase (PDB ID 1W6J) was downloaded from the protein data bank (http://www.rcsb.org/pdb), which is a complex catalytic portion of human HMGCoA reductase with HMGCoA. We obtained PDBIDs 1DQ9,

1DQ8, 1DQA for HMGCoA reductase. We obtained 1W6J, 1W6K for Lanosterol synthase. Among them we selected 1DQ9 for HMGCoA reductase and 1W6J for Lanosterol synthase as they have maximum resolution among others and low polymers and ligands attached to it. We then download the required files

5.2.2 SELECTION OF LIGAND MOLECULE FOR HMGCOA REDUCTASE AND LANOSTEROL SYNTHASE:

We searched for different natural treatments which are known to lower cholesterol in our body. We studied the ancient method of treatment for heart diseases. We investigated different antioxidant foods, natural molecules and listed them. We also found that a class of Quinuclidine inhibitors [6] are known to inhibit Lanosterol synthase. We then opened PUBCHEM and searched for the structure and files required for different molecules mentioned above. We downloaded the required .sdf. We later converted the file into .pdb and .pdbqt.

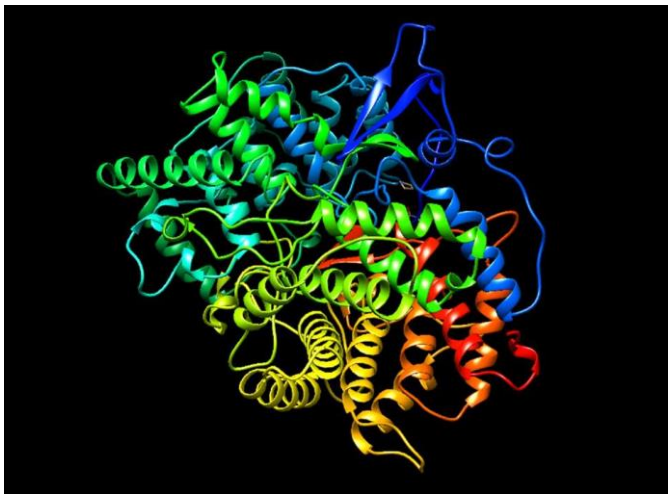


Figure 3: Crystallographic structure of Lanosterol synthase with PDB ID **1W6J**

Figure 4: Crystallographic structure of HMGCoA reductase with PDB ID **1DQ9**

5.2.3 PREDICTION OF ACTIVE SITE OF PROTEIN:

Active sites of HMGCoA reductase and Lanosterol synthase were predictes using CASTP. We open http://sts-fw.bioengr.uic.edu/castp/calculation.php in our taskbar. We submit the PDBID of required protein in the search box. We obtain different amino acid residues present at the active site of our required protein. For HMGCoA reductase we found four residues GLU559, LYS691, ASP767 and HIS866 as catalytic portions of our active site. We later analyzed the

catalytic aminoacid chains using UNIPROT. We then predicted the binding pockets using CASTP. We found HIS232, ASP455 as amino acid residues at active site for Lanosterol synthase.

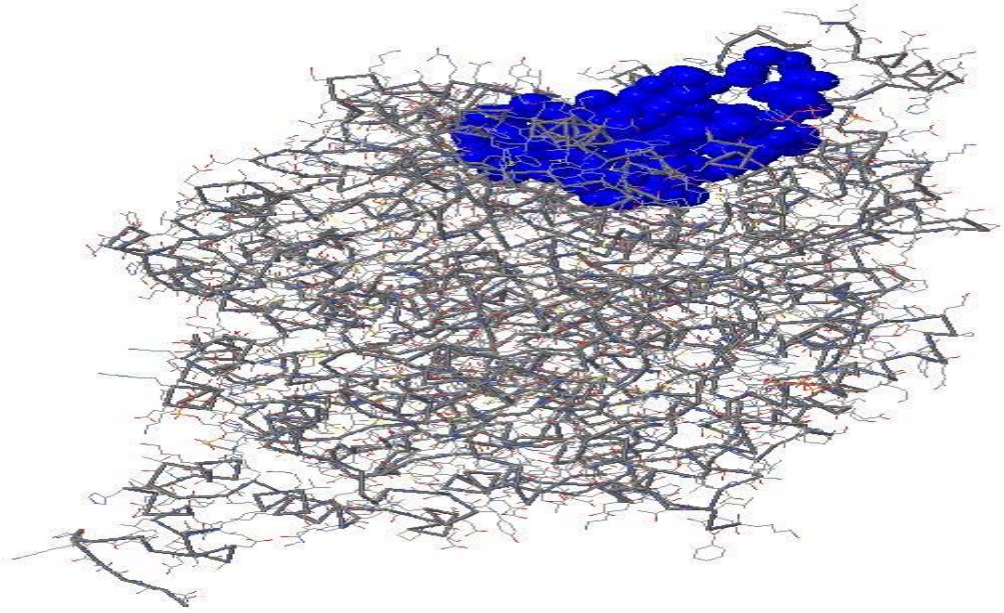


Figure 5: CASTP analysis of Active Sites of HMGCoA Reductase with PDB ID 1DQ9, showing 559 Amino acid residue of catalytic portion

5.2.4 DOCKING:

Docking is a procedural method to predict the preferred orientation of one molecule to another when bound forming a stable complex. Docking [11] is important in Drug designing which is used to calculating the binding alignment of small molecular drugs or inhibitors to their protein targets and can predict affinity and activity of complex formed. Docking is important in rational drug designing. Receptor and ligand are important for this docking study. Receptor is a receiving molecule mostly a protein. Ligand is its complementary partner which binds to receptor forming a complex.

In this study we have used Arguslab, Mgl tools, Autodock vina dock engine for obtaining our results

5.2.5 SELECTION OF TARGET PROTEIN:

We used http://www.rcsb.org/pdb/home/home.do to choose the target protein file. We obtained PDBIDs 1DQ9, 1DQ8, 1DQA for HMGCoA reductase. We obtained 1W6J, 1W6K for Lanosterol synthase. Among them we selected 1DQ9 for HMGCoA reductase and 1W6J for Lanosterol synthase as they have maximum resolution among others and low polymers and ligands attached to it. We then download the required files.

5.2.6 VISUALIZATION OF MOLECULE USING PYMOL:

With the help of PYMOL, we visualize the molecules so that we can obtain better quality 3D images of all molecules (small, biological, large etc).

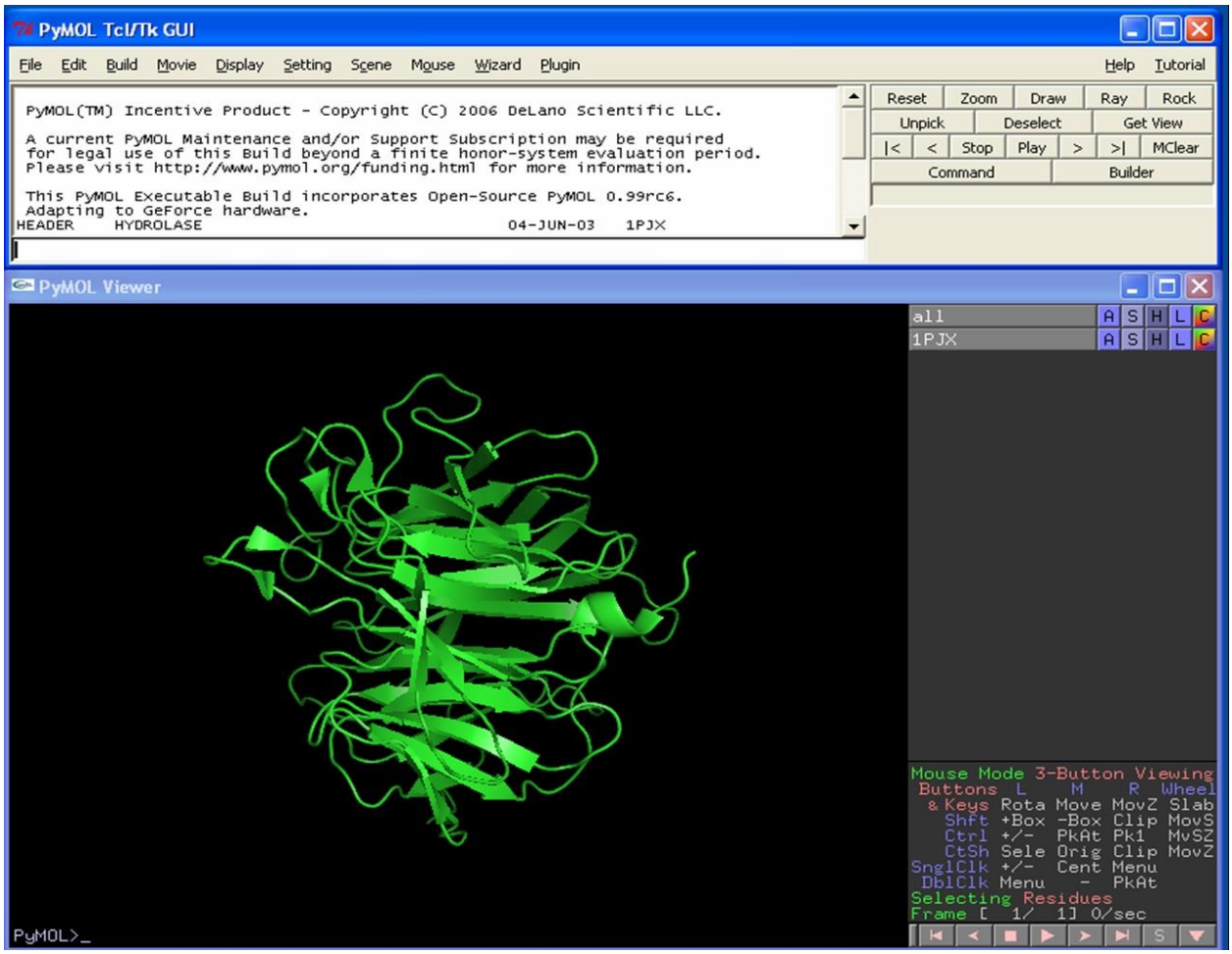


Figure 6: Example for PYMOL visualization of Biological molecules

5.2.7 TOXICITY PREDICTION USING CHEMBIOSERVER:

Select the required molecule in .pdb and open it in Ibabel. Convert the .pdb file into .mol in Ibabel and save it. Now Chembioserver website was opened.

- Open " Toxicity filtering "
- Select the "choose file" and choose the .mol file.
- Press " Proceed data " button below
- Download the toxicity results file.

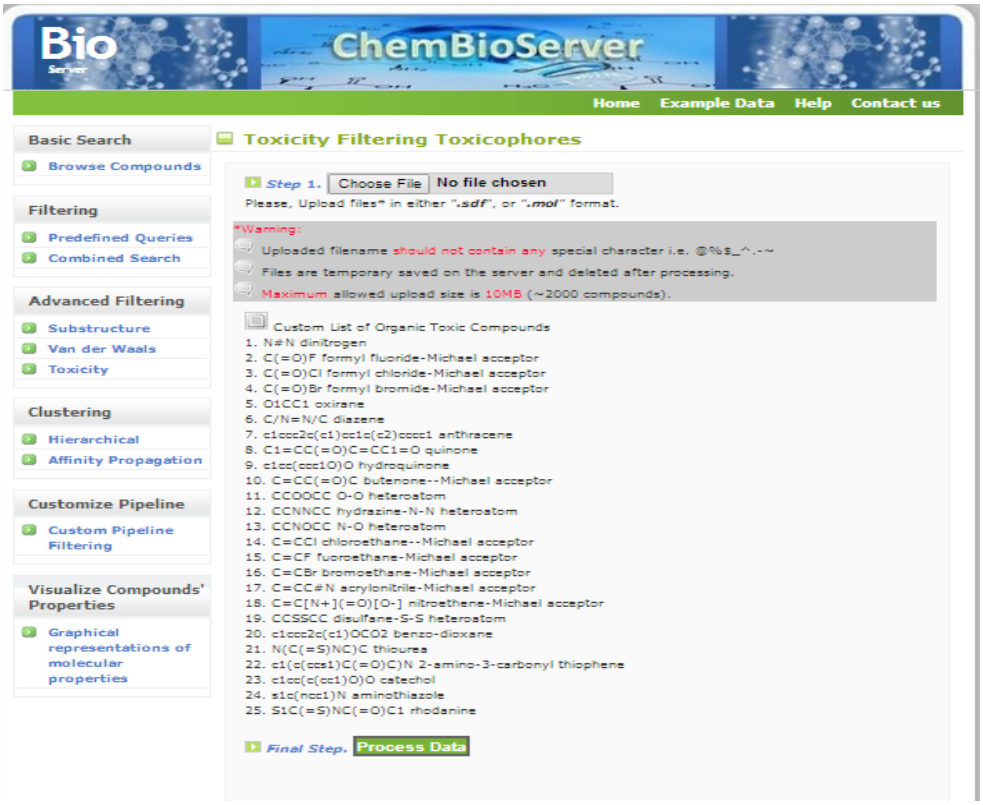


Figure 7: Shows the list of Organic Toxic compounds in ChemBioServer Toxicity prediction server

The online server "Chembioserver" recognizes a list of organic toxic compounds. This server checks if the molecule contains any of organic toxic compounds. If it does not contain any of the organic toxic compounds, the result is PASS i.e Nontoxic. If it contains any toxic compounds, the result is FAIL i.e Toxic.

5.2.8 DRUGLIKENESS OF OUR SELECTED INHIBITORS

Druglikeness is used in drug designing to know how druglike a molecule or substance is considering factors like bioavailability. Properties of druglike molecule include solubility, molecular weight, molar refractivity, pharmacological properties, ligand efficiency, lipophilic efficiency. Partition coefficient. We used http://www.chemicalize.org/ to find out different properties for analysis of Druglikeness properties.

CHAPTER 6

RESULTS

6 RESULTS

6.1 PYMOL VIZUALIZATION OF STATINS:

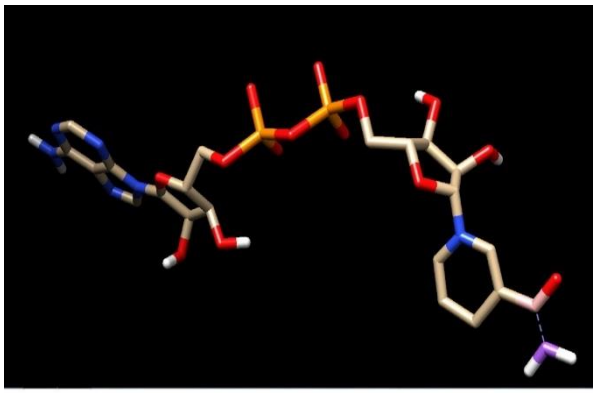


Figure 8: Visualized Structure of Pravastatin (Drug Bank ID DB00175) in PYMOL

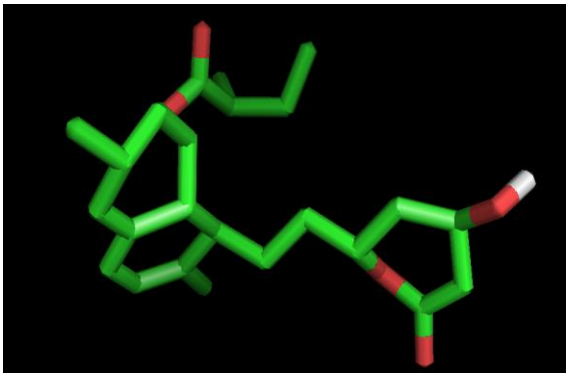


Figure 9: Visualized Structure of Lovastatin (Drug Bank ID DB00227) in PYMOL

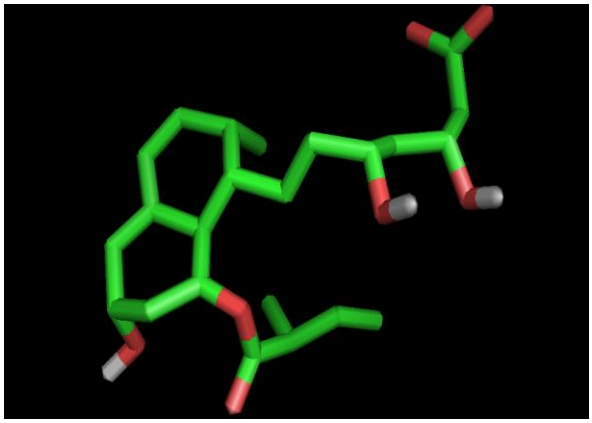


Figure 10: Visualized Structure of NADH (Drug Bank ID DB00157) in PYMOL

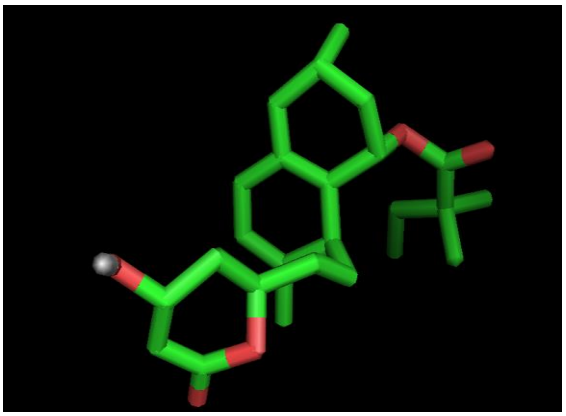


Figure 11: Visualized Structure of Simvastatin (Drug Bank ID DB00641) in

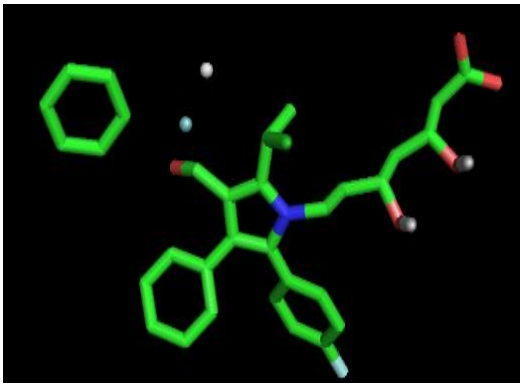


Figure 12: Visualized Structure of Atorvavastatin (Drug Bank ID DB01076) in PYMOL

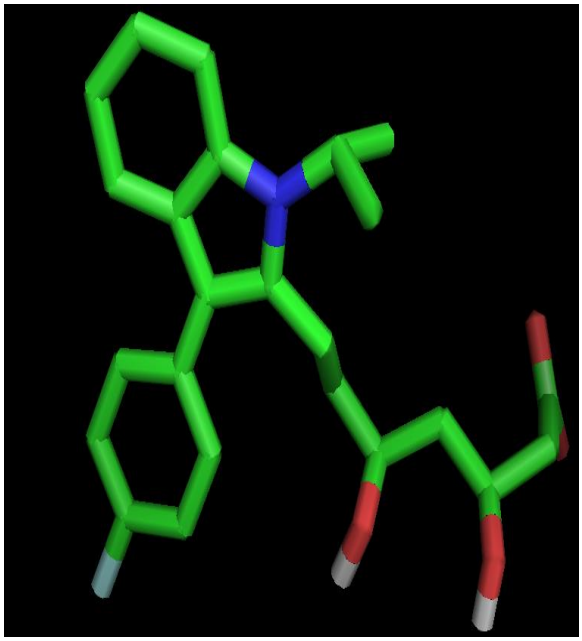


Figure 13: Visualized Structure of Fluvastatin (Drug Bank ID DB01095) in PYMOL

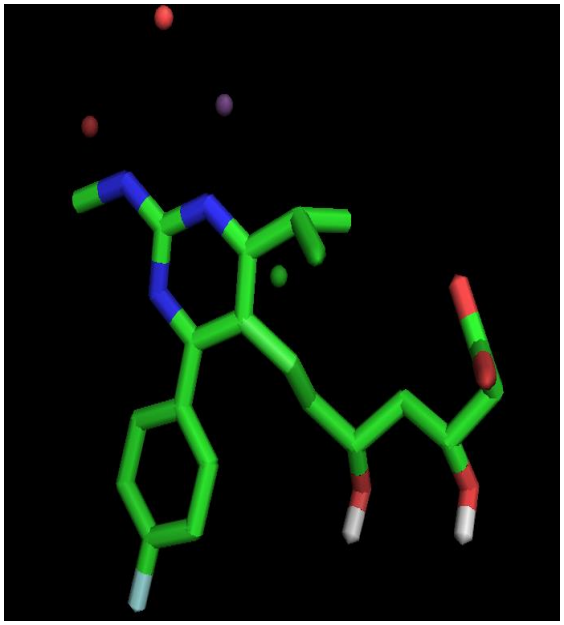


Figure 14: Visualized Structure of Rosuvastatin (Drug Bank ID DB01098) in PYMOL

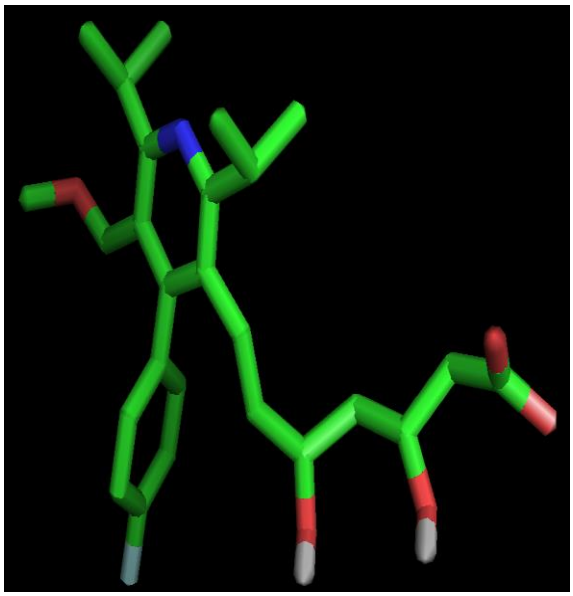


Figure 15: Visualized Structure of Serivastatin (Drug Bank ID DB00439) in PYMOL

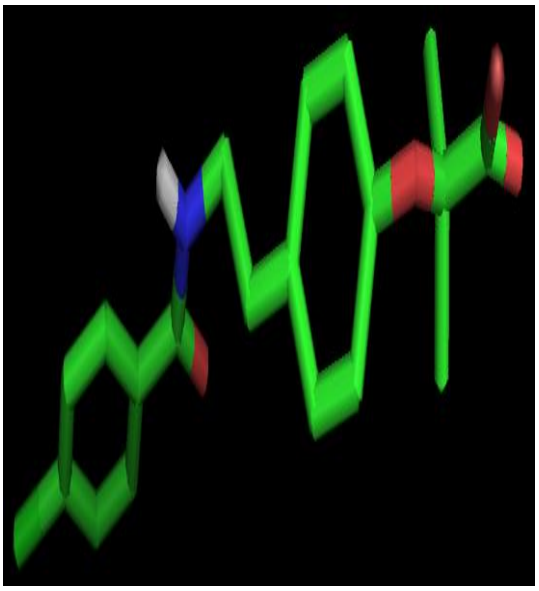


Figure 16: Visualized Structure of Bezafibrate (Drug Bank ID DB01393) in PYMOL

6.2 PYMOL VIZUALIZATION OF NATURAL INHIBITORS:

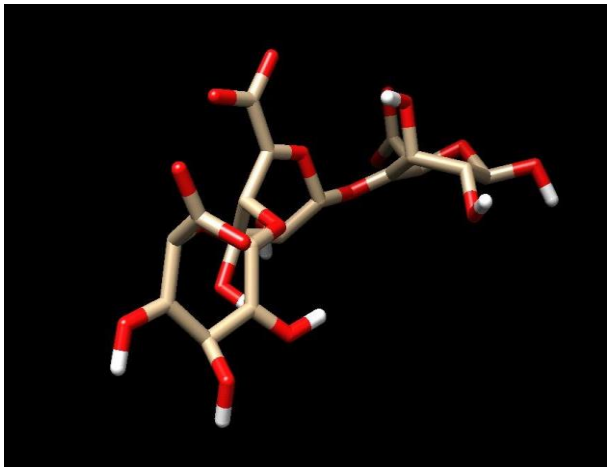


Figure 17: Visualization of pectin using PYMOL

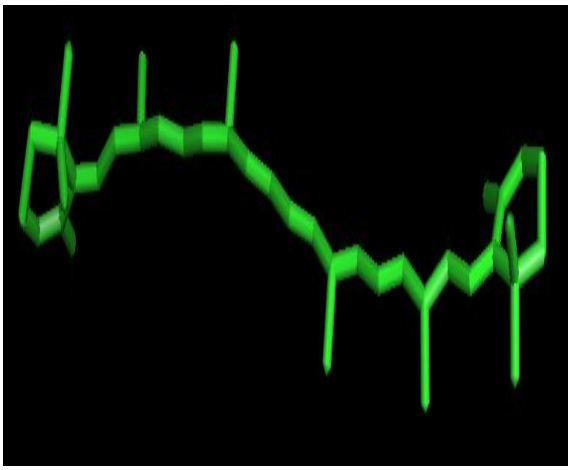


Figure 18: Visualization of beta Carotene using PYMOL

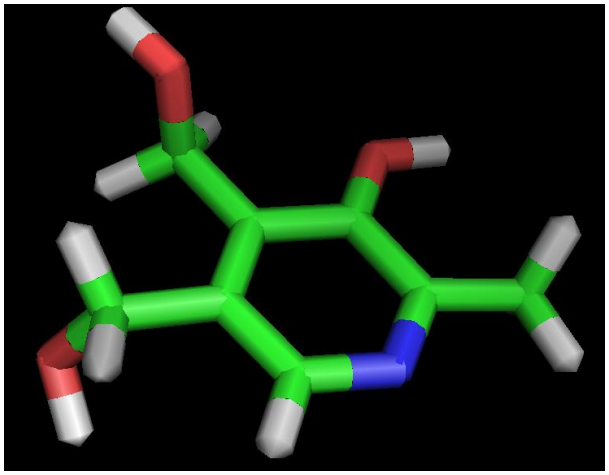


Figure 19: Visualization of Riboflavin using PYMOL

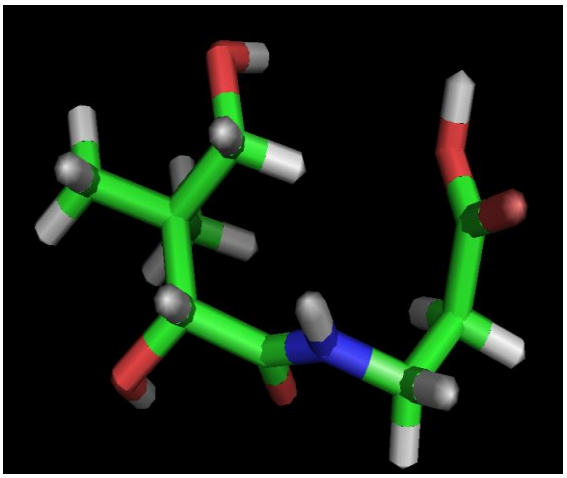


Figure 20: Visualization of Pantothenic acid using PYMOL

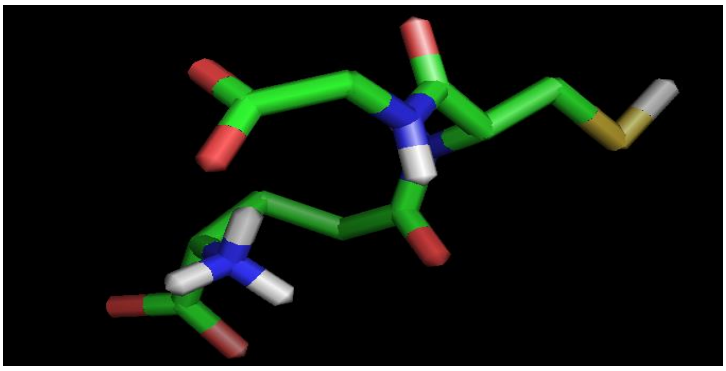


Figure 21: Visualization of Glutathione using PYMOL

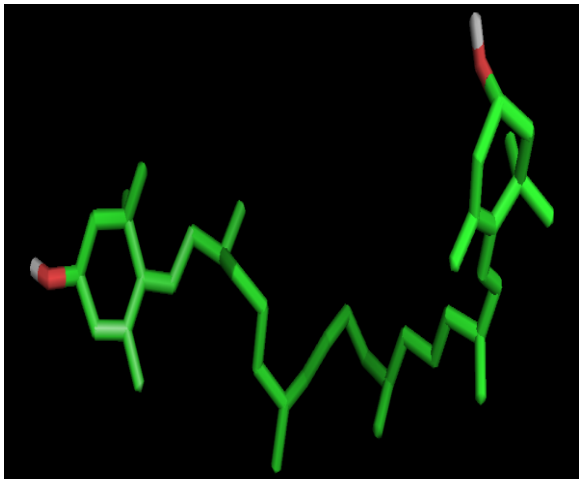


Figure 22: Visualization of Lutein using PYMOL

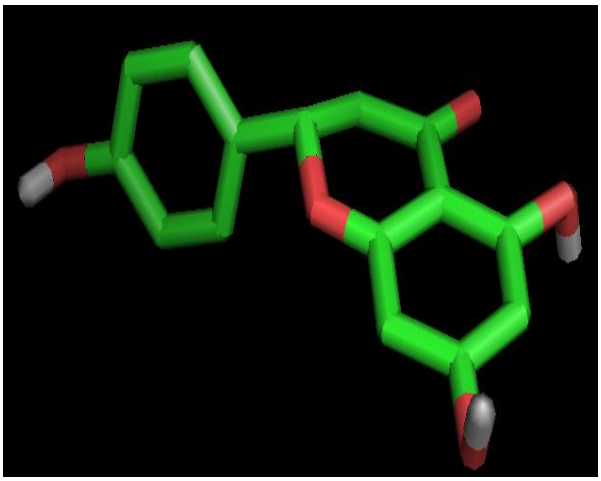


Figure 23: Visualization of Naringenin using PYMOL

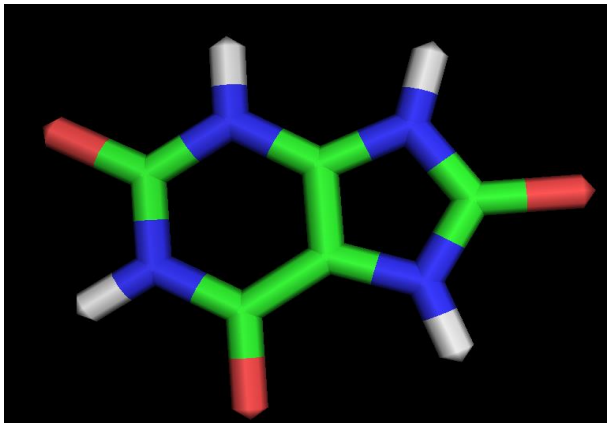


Figure 24: Visualization of Uric acid using PYMOL

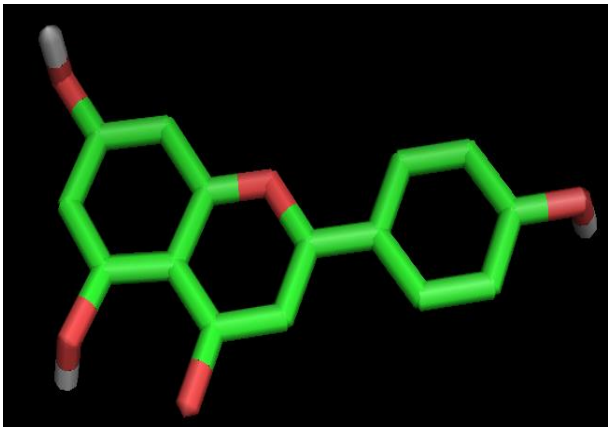


Figure 25: Visualization of Apigenin using PYMOL

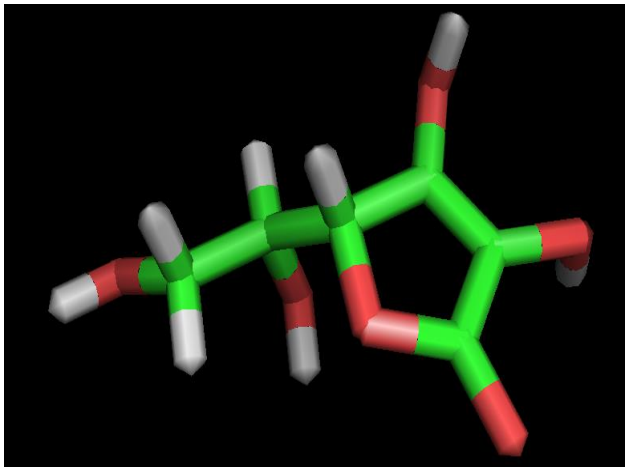


Figure 26: Visualization of Ascorbic acid using PYMOL

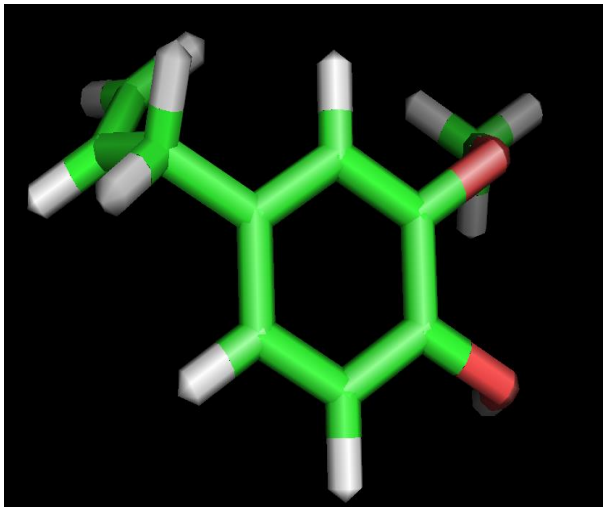


Figure 27: Visualization of Eugenol using PYMOL

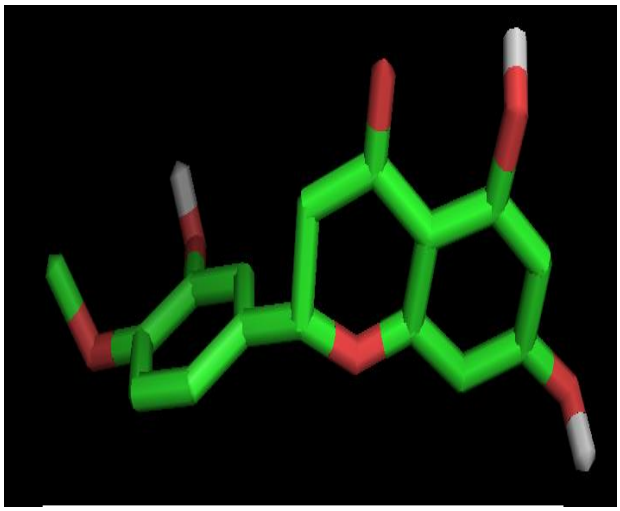


Figure 28: Visualization of Hesperetin using PYMOL

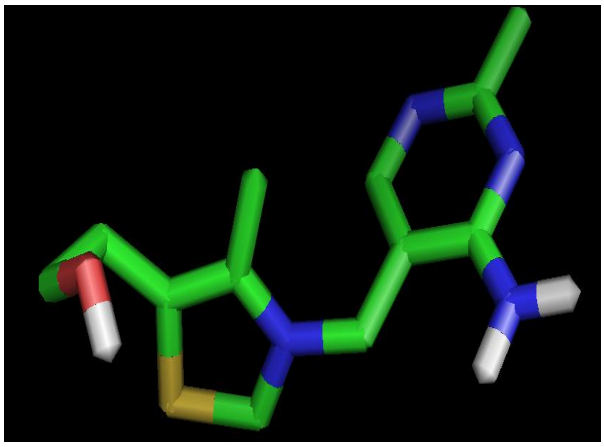


Figure 29: Visualization of Thiamine using PYMOL

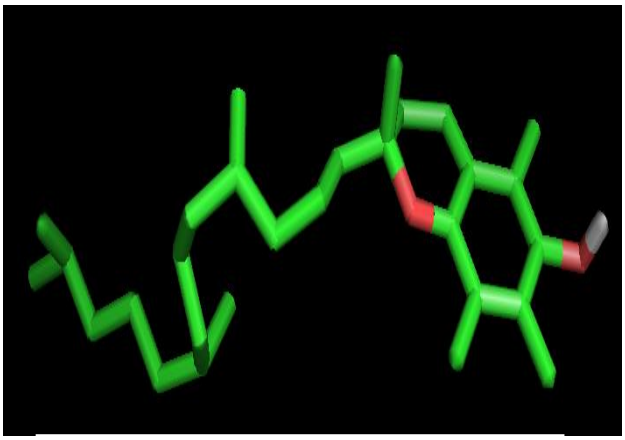


Figure 30: Visualization of Vitamin E using PYMOL

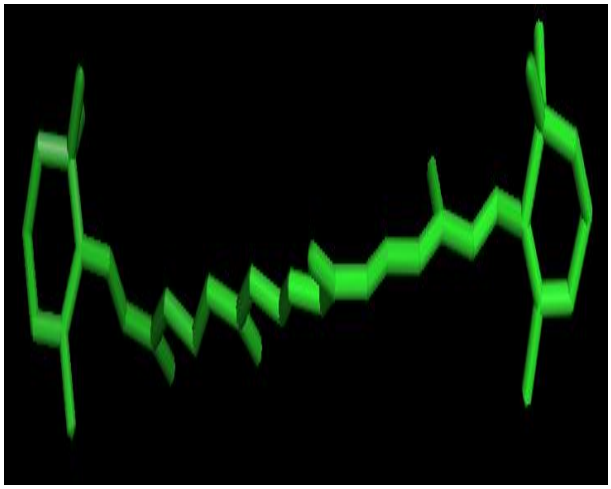


Figure 31: Visualization of alpha Carotene using PYMOL

6.3 PYMOL VIZUALIZATION OF QUINUCLIDINE INHIBITORS:

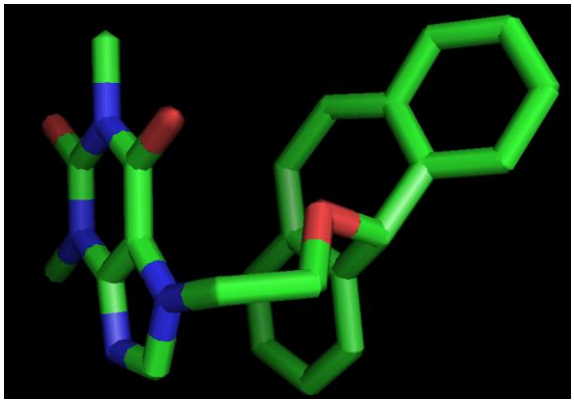


Figure 32: Visualization of CID 1006318 using PYMOL

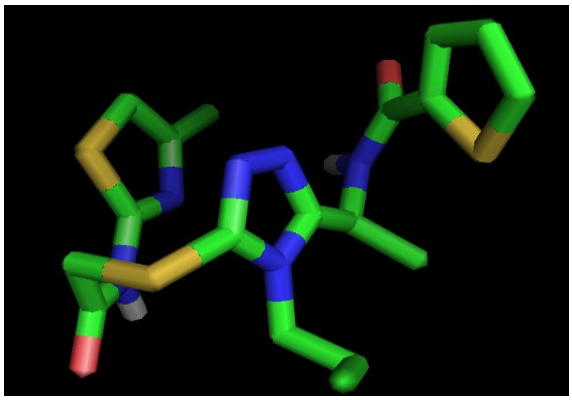


Figure 33: Visualization of CID 2213568 using PYMOL

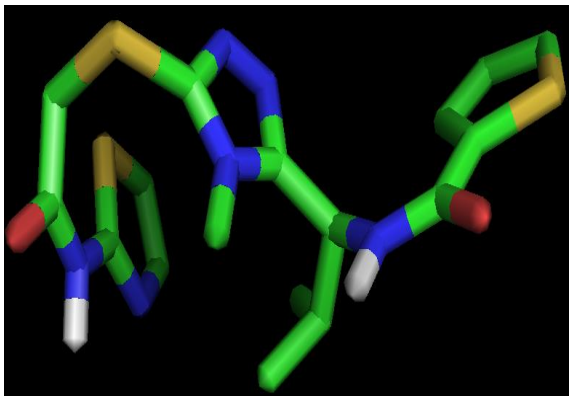


Figure 34: Visualization of CID 2213595 using PYMOL

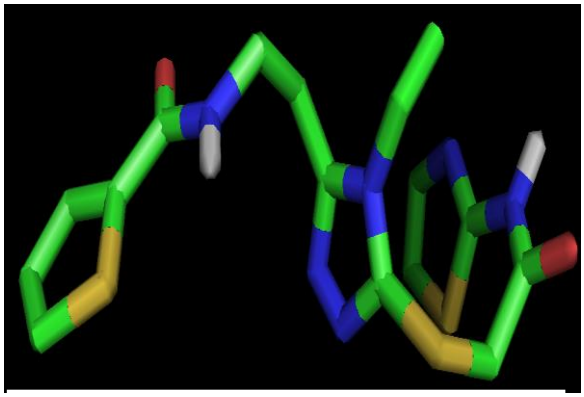


Figure 35: Visualization of CID 2213690 using PYMOL

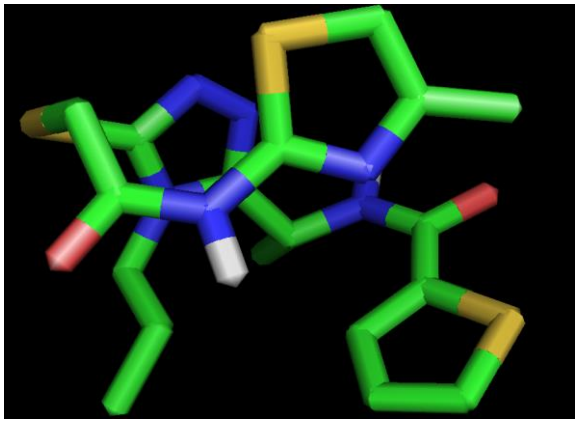


Figure 36: Visualization of CID 2958449 using PYMOL

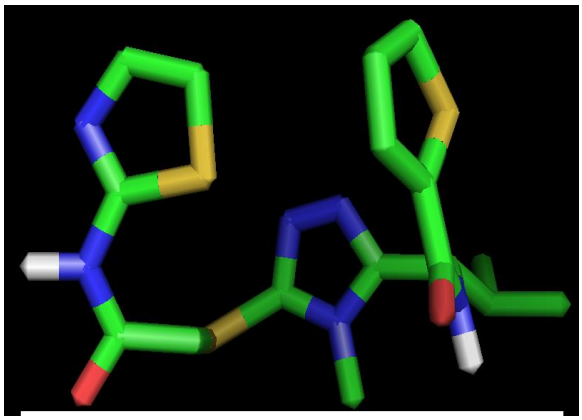


Figure 37: Visualization of CID 2958467 using PYMOL

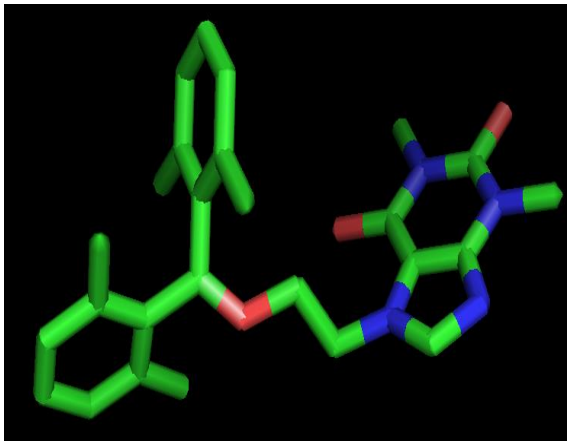


Figure 38: Visualization of CID 3428244 using PYMOL

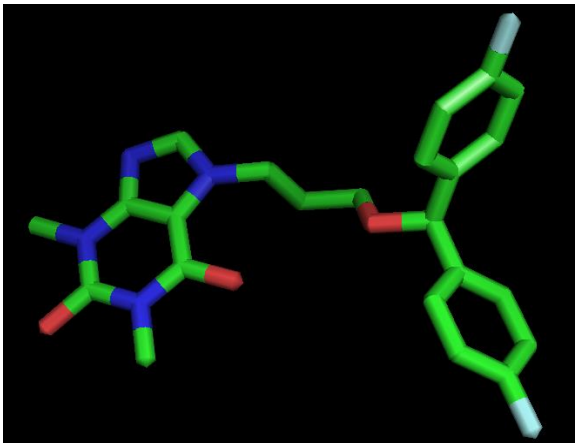


Figure 39: Visualization of CID 4392376 using PYMOL

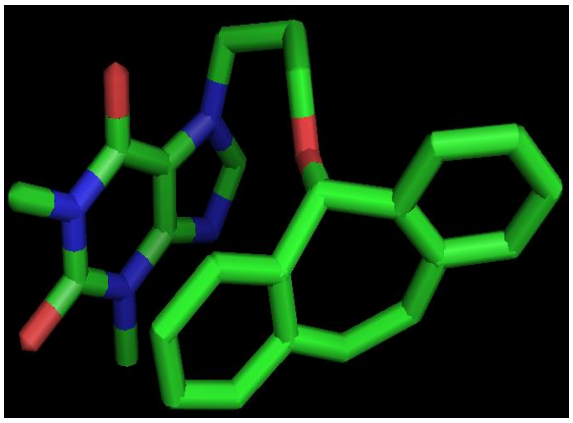


Figure 40: Visualization of CID 4398863 using PYMOL

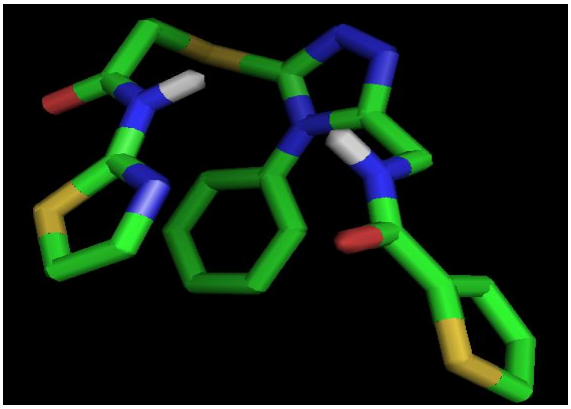


Figure 41: Visualization of CID 4617056 using PYMOL

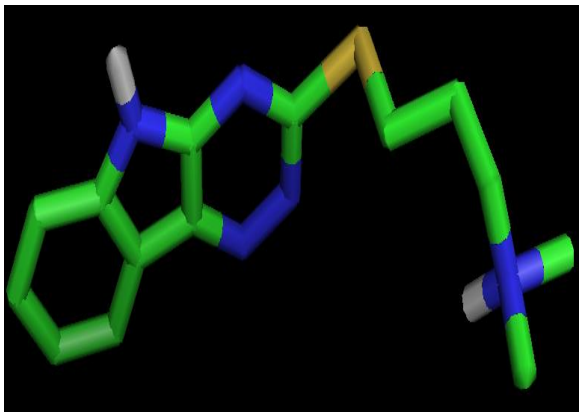


Figure 42: Visualization of CID 6413427 using PYMOL

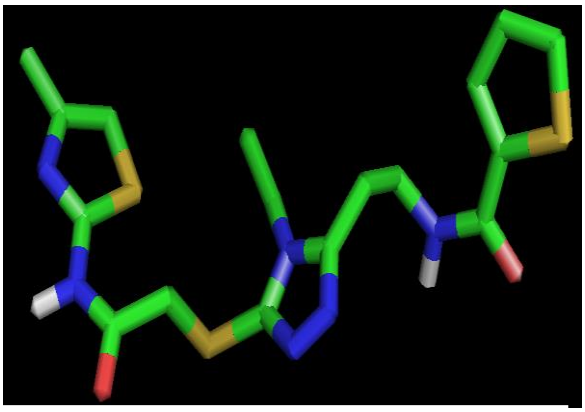


Figure 43: Visualization of CID 9542937 using PYMOL

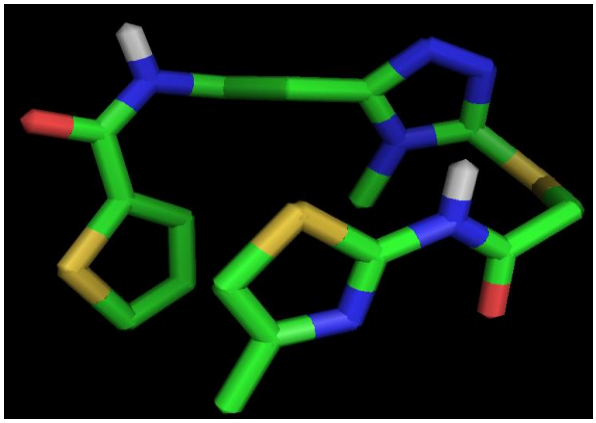


Figure 44: Visualization of CID 9542938 using PYMOL

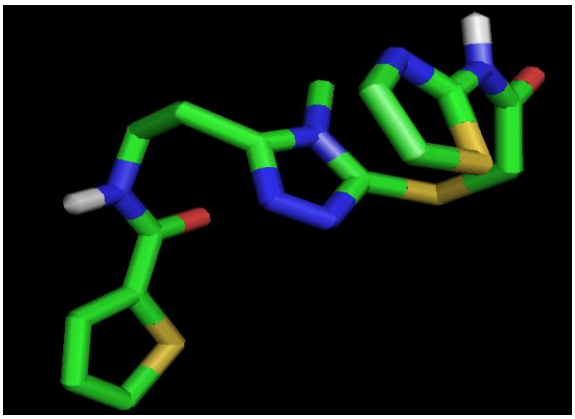


Figure 45: Visualization of CID 9542939 using PYMOL

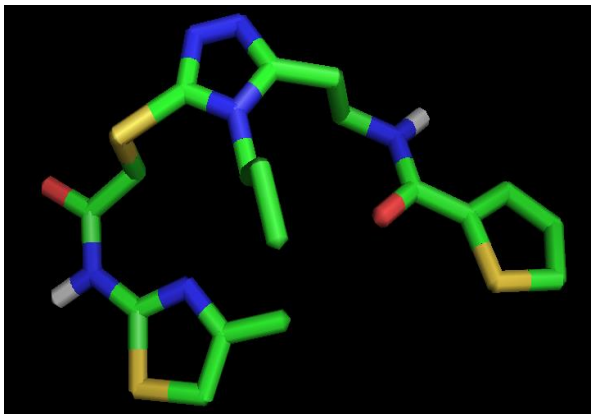


Figure 46: Visualization of CID 9542940 using PYMOL

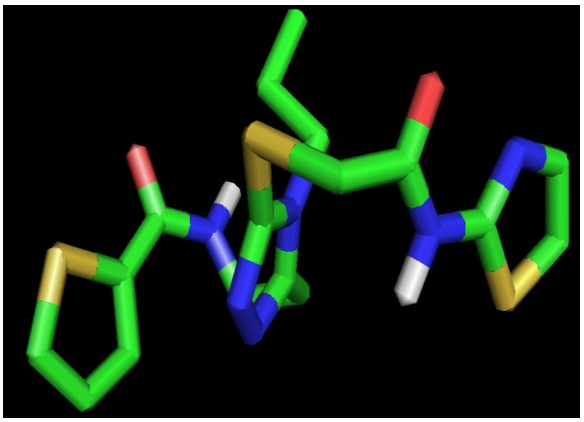


Figure 47: Visualization of CID 9542941 using PYMOL

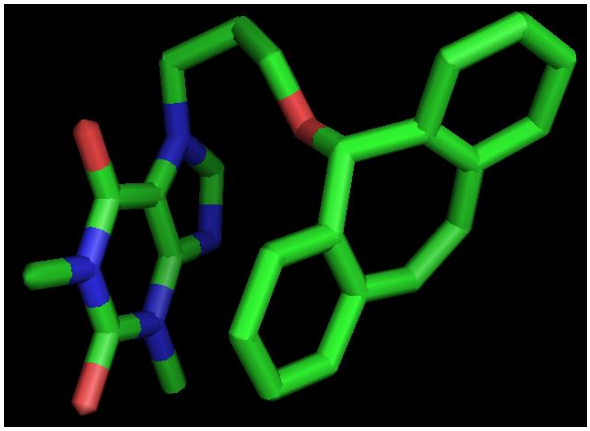


Figure 48: Visualization of CID 11838789 using PYMOL

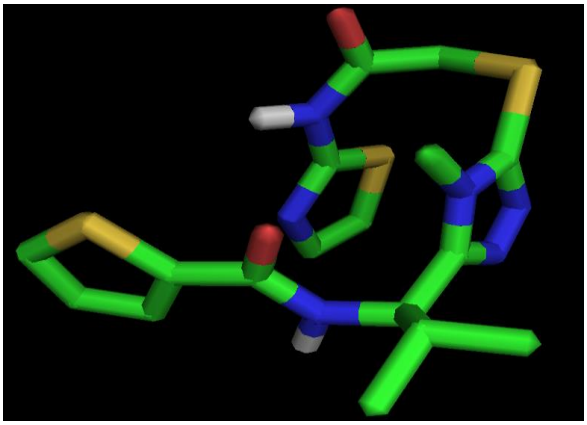
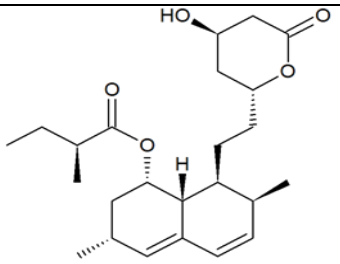
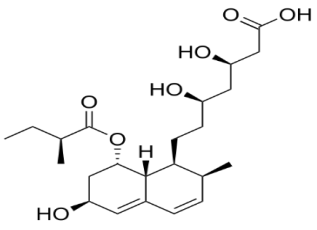
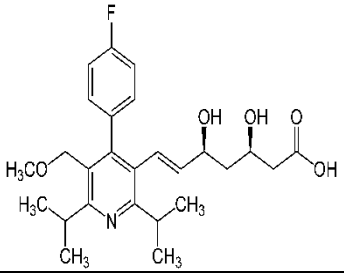
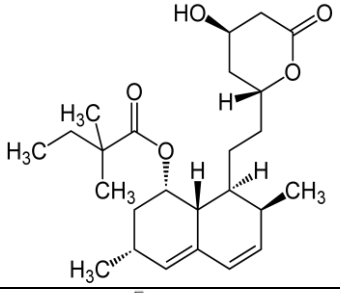
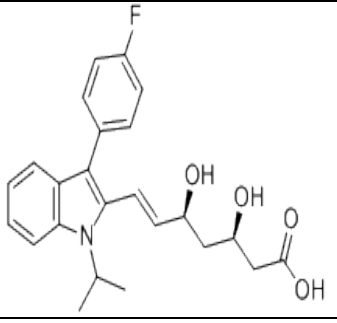
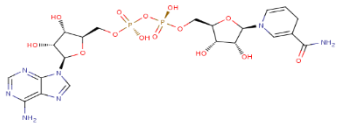
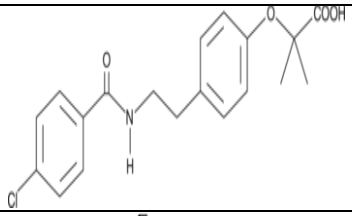
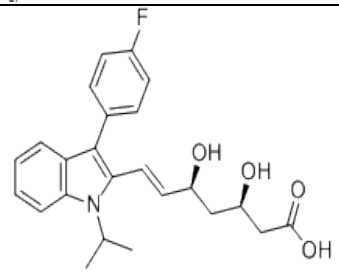
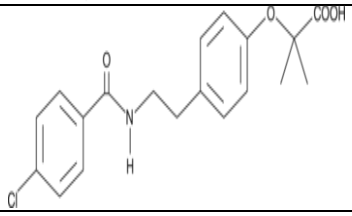
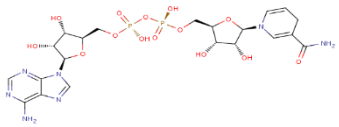
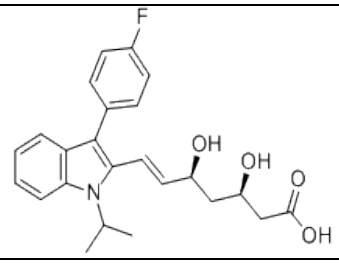
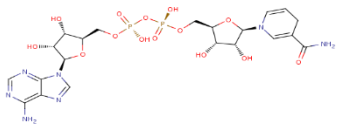


Figure 49: Visualization of CID 40478367 using PYMOL

6.4 POSITIVE CONTROL 1:

Table A: Binding energy results obtained on docking HMGCoA reductase with available drugs

Sl No	Name of the Compound	Drug Bank ID	Structure	Binding Energy (K.cal/mol)	Active site residue
1	Lovastatin	DB00227		-7.66	559 catalytic portion
2	Pravastatin	DB00175		-6.2	559 catalytic portion
3	Cerivastatin	DB00439		-6.8	559 catalytic portion
4	Simvastatin	DB00641		-7.2	559 catalytic portion
5	Fluvastatin	DB01095		-7.3	559 catalytic portion

6	**NADH**	**DB00157**		**-7.8**	**559 catalytic portion**
7	**Bezafibrate**	**DB01393**		**-6.7**	**559 catalytic portion**
8	**Fluvastatin**	**DB01095**		**-5.3**	**691 catalytic portion**
9	**Bezafibrate**	**DB01393**		**-5.6**	**691 catalytic portion**
10	**NADH**	**DB00157**		**-7.8**	**767 catalytic portion**
11	**Fluvastatin**	**DB01095**		**-7.1**	**767 catalytic portion**
12	**NADH**	**DB00157**		**-7.7**	**866 catalytic portion**

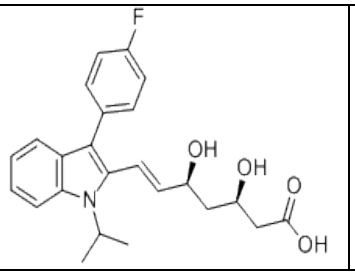
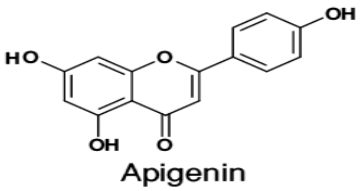
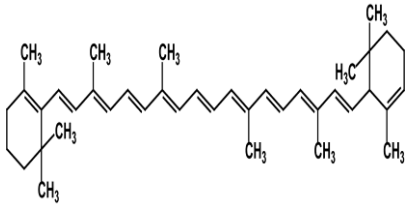
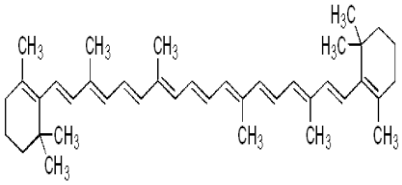
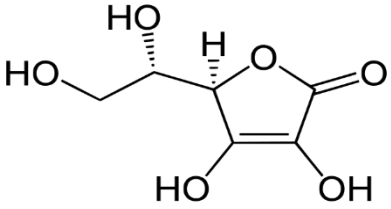
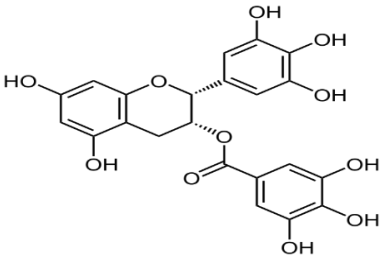
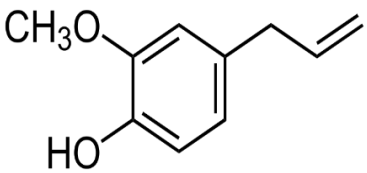
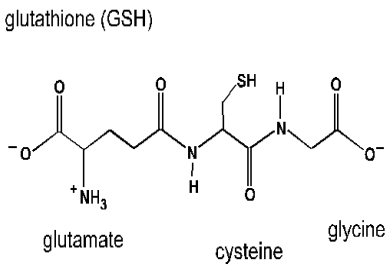
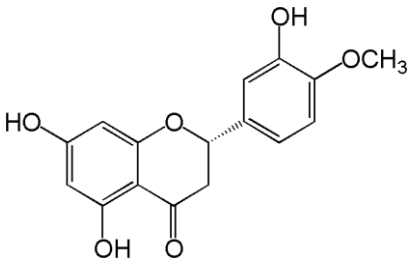
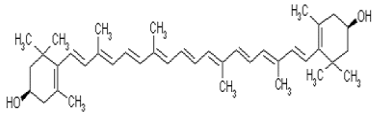
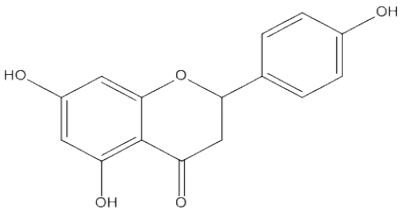
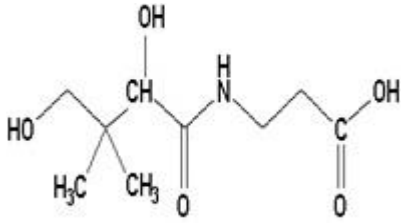
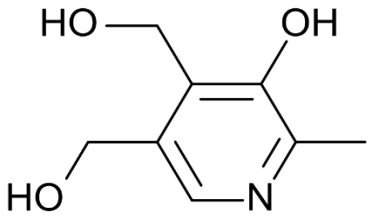
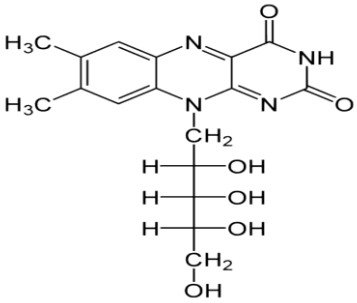
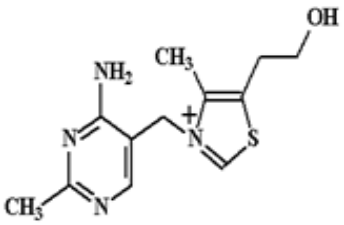
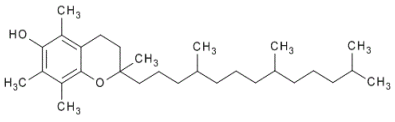
13	**Fluvastatin**	**DB01095**		**-6.9**	**866 catalytic portion**

Table B: Binding Energy results obtained on Docking HMGCoA reductase with natural molecules at *767 Catalytic portion of active site*

Sl No	Name of the natural Compound	Structure	Source	Binding Energy (K.cal/mol)
1	**Apigenin**	 Apigenin	**Vegetables, fruits, spices etc**	**-7.2**
2	**α-carotene**		**Fruits, carrots, vegetables**	**-6.5**
3	**β-carotene**		**Carrots, sweet potato etc**	**-7.1**
4	**Ascorbic acid**		**Citrus fruits**	**-5.2**

5	**Epigallo catechin Gallate**		**Green tea, fruits, nuts**	**-7.7**
6	**Eugenol**		**Herbs, spices**	**-5.0**
7	**Glutathione**		**Garlic, onions etc**	**-5.7**
8	**Hesperetin**		**Citrus fruits**	**-7.3**
9	**Lutein**		**Plants and leafy vegetables**	**-6.6**
10	**Naringenin**		**Citrus fruits**	**-7.3**

11	**Pantothenic acid**		**Yoghurt, eggs, meat etc**	**-5.6**
12	**Pyridoxine**		**Fish, meat, liver etc**	**-4.9**
14	**Riboflavin**		**Leafy foods, mushrooms, liver, milk etc**	**-4.9**
15	**Thiamine**		**Eggs, liver, chicken, mutton etc**	**-6.4**
16	**Tocoferol**		**Nuts, seeds, vegetable oils etc**	**-6.7**

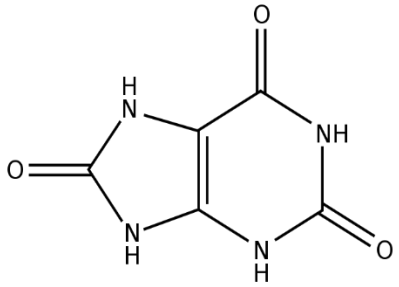
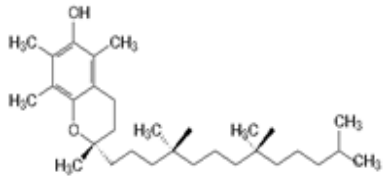
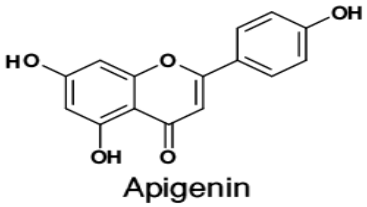
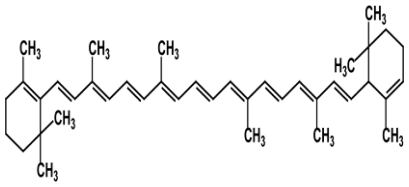
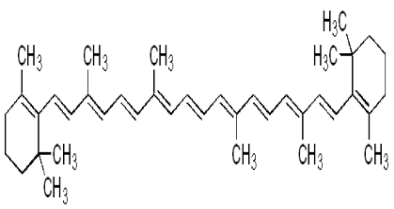
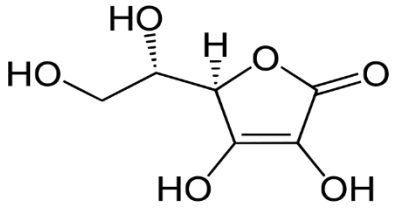
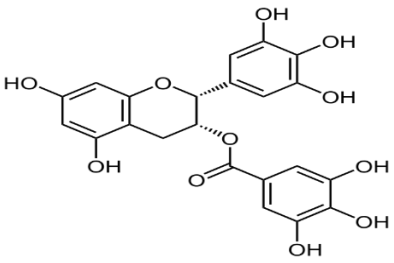
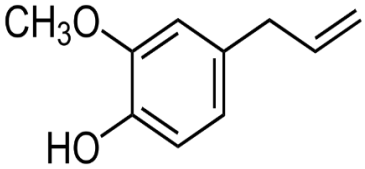
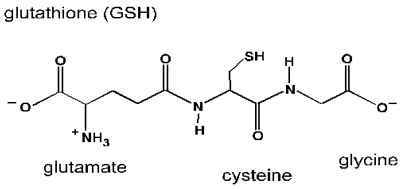
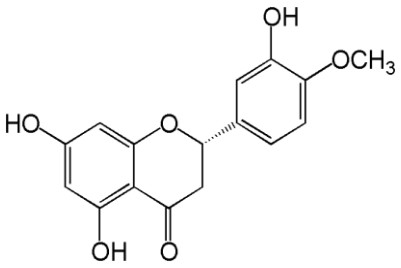
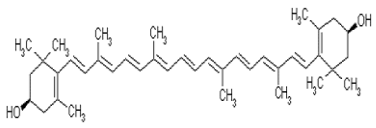
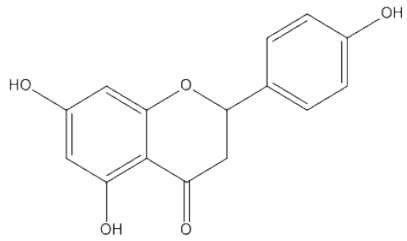
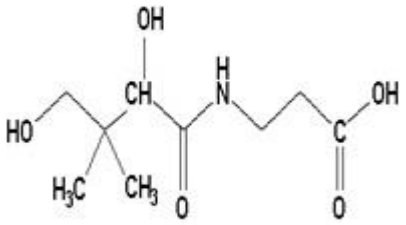
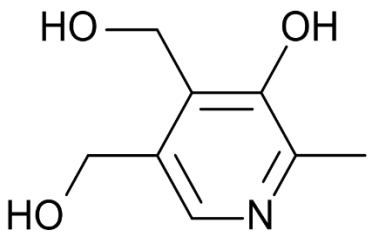
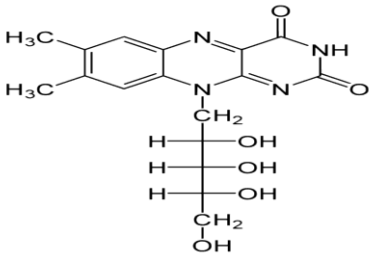
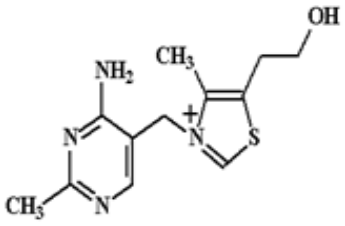
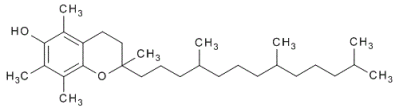
17	**Uric acid**		**Seafoods, meat etc**	**-5.6**
18	**Vitamin E**		**Sunflower oil, almond, papaya etc**	**-6.8**

Table C: Binding Energy results obtained on Docking HMGCoA reductase with natural molecules at *866 Catalytic portion of active site*

Sl No	Name of the natural Compound	Structure	Source	Binding Energy (K.cal/mol)
1	**Apigenin**	 Apigenin	**Vegetables, fruits, spices etc**	**-6.4**
2	**α-carotene**		**Fruits, carrots, vegetables**	**-4.0**
3	**β-carotene**		**Carrots, sweet potato etc**	**-5.4**

4	**Ascorbic acid**		**Citrus fruits**	**-5.1**
5	**Epigallo catechin Gallate**		**Green tea, fruits, nuts**	**-7.1**
6	**Eugenol**		**Herbs, spices**	**-4.9**
7	**Glutathione**		**Garlic, onions etc**	**-5.5**
8	**Hesperetin**		**Citrus fruits**	**-6.9**
9	**Lutein**		**Plants and leafy vegetables**	**-5.1**

10	**Naringenin**		**Citrus fruits**	**-6.6**
11	**Pantothenic acid**		**Yoghurt, eggs, meat etc**	**-5.2**
12	**Pyridoxine**		**Fish, meat, liver etc**	**-5.0**
13	**Riboflavin**		**Leafy foods, mushrooms, liver, milk etc**	**-5.0**
14	**Thiamine**		**Eggs, liver, chicken, mutton etc**	**-5.9**
15	**Tocoferol**		**Nuts, seeds, vegetable oils etc**	**-5.5**

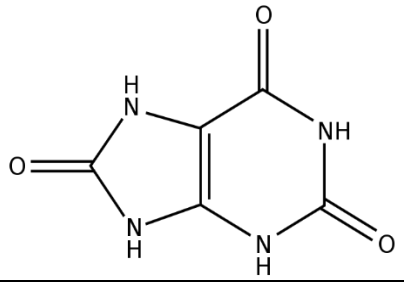
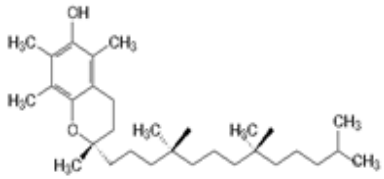
16	**Uric acid**		**Seafoods, meat etc**	**-5.3**
17	**Vitamin E**		**Sunflower oil, almond, papaya etc**	**-5.3**

Table D: Toxicity and Drug likeness of above natural compounds

Sl No	Name of the natural Compound	Toxicity Result	Log P Value (partition co-efficient)
1	**Apigenin**	**Non toxic**	**2.71**
2	**Ascorbic acid**	**Non toxic**	**-1.91**
3	**Epigallo catechin Gallate**	**Non toxic**	**3.08**
4	**Eugenol**	**Non toxic**	**2.61**
5	**Glutathione**	**Non toxic**	**-4.68**
6	**Hesperetin**	**Non toxic**	**2.68**
7	**Lutein**	**Non toxic**	**8.55**
8	**Naringenin**	**Non toxic**	**2.64**
9	**Pantothenic acid**	**Non toxic**	**-1.36**
10	**Pyridoxine**	**Non toxic**	**-0.95**
11	**Riboflavin**	**Non toxic**	**-0.92**
12	**Thiamine**	**Non toxic**	**-3.10**
13	**Tocoferol**	**Non toxic**	**10.51**
14	**Uric acid**	**Non toxic**	**-1.54**

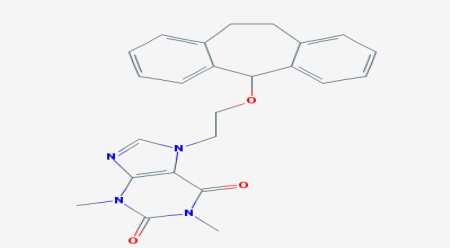
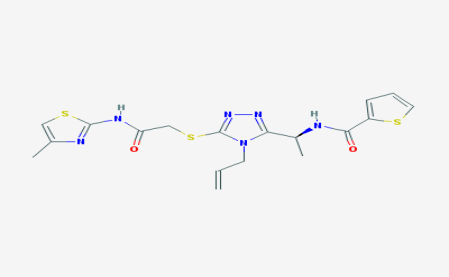
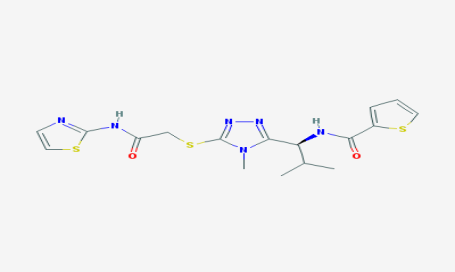
Table E: Log P value of Statins

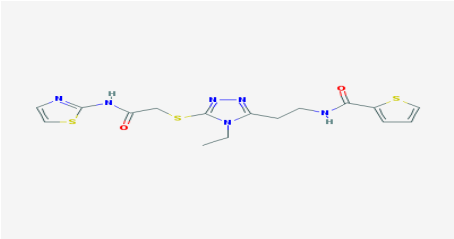
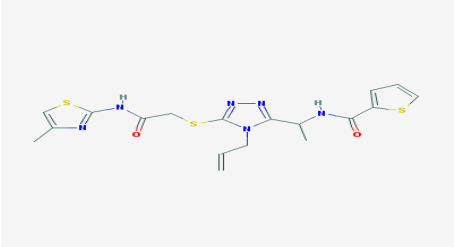
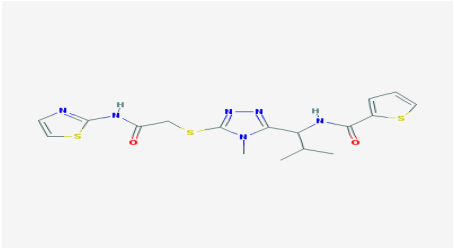
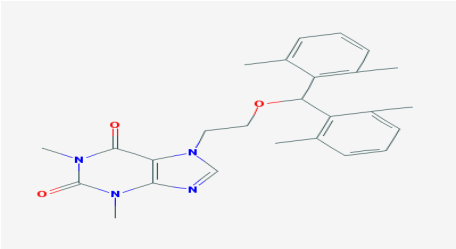
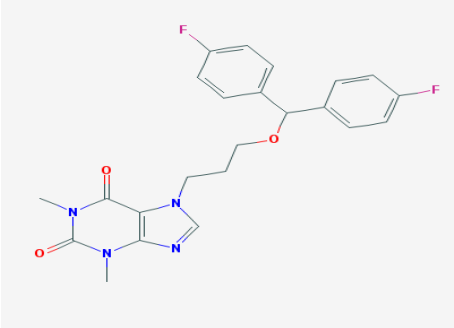
Sl No	Compound	Log P value
1	**Pravastatin**	**1.65**
2	**Lovastatin**	**3.90**

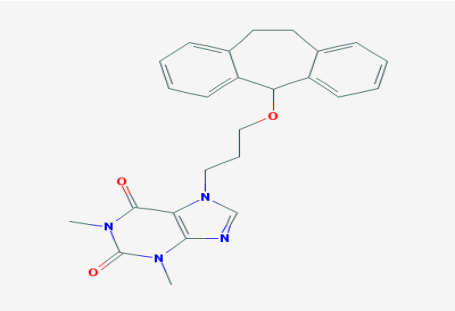
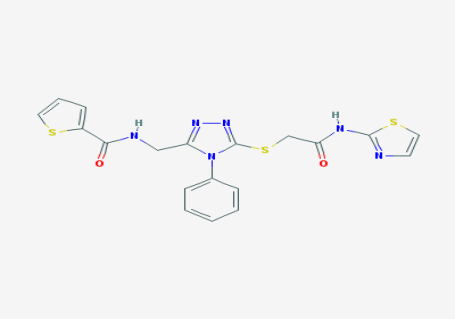
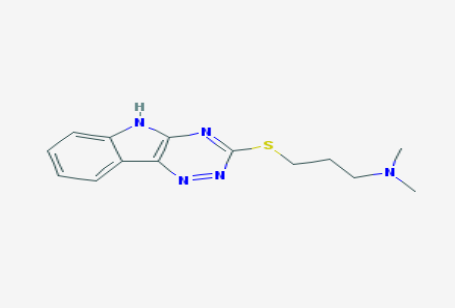
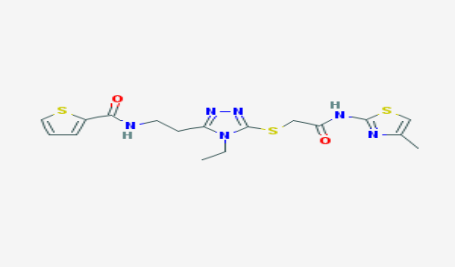
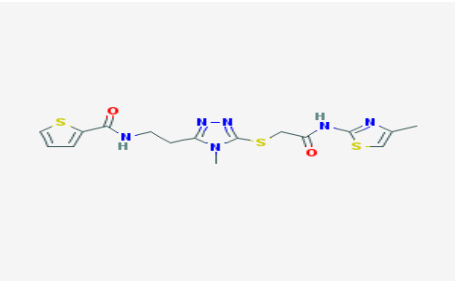
3	Ceravastatin	2.67
4	Simvastatin	4.46
5	Fluvastatin	3.83
6	Atorvastatin	5.39
7	Mevastatin	3.62
8	Rosuvastatin	1.92

6.5 POSITIVE CONTROL 2:

Table F: Binding energy results obtained on docking Lanosterol synthase with identified class of QUINUCLIDINE INHIBITORS and related structures inhibiting Lanosterol synthase.

Sl No	PUBCHEM ID	Structure	Molecular Weight	Binding Energy (k.cal/mol)	Active site catalytic portion
1	CID 1006318		416.47	-6.4	HIS 232
				-8.3	ASP 455
2	CID 2213568		448.58	-5.7	HIS 232
				-6.1	ASP 455
3	CID 2213595		436.57	-5.8	HIS 232
				-7.0	ASP 455

4	**CID 2213690**		**422.54**	**-5.6**	**HIS 232**
				-7.1	**ASP 455**
5	**CID 2958449**		**448.58**	**-5.1**	**HIS 232**
				-6.7	**ASP 455**
6	**CID 2958467**		**436.57**	**-6.1**	**HIS 232**
				-6.0	**ASP 455**
7	**CID 3428244**		**446.54**	**-6.8**	**HIS 232**
				-7.6	**ASP 455**
8	**CID 4392376**		**440.44**	**-7.1**	**HIS 232**
				-7.7	**ASP 455**

9	**CID 4398863**		**430.49**	**-7.5**	**HIS 232**
				-8.4	**ASP 455**
10	**CID 4617056**		**456.56**	**-6.8**	**HIS 232**
				-7.7	**ASP 455**
11	**CID 6413427**		**287.38**	**-7.7**	**HIS 232**
				-6.4	**ASP 455**
12	**CID 9542937**		**436.57**	**-5.8**	**HIS 232**
				-6.2	**ASP 455**
13	**CID 9542938**		**422.54**	**-6.8**	**HIS 232**
				-7.3	**ASP 455**

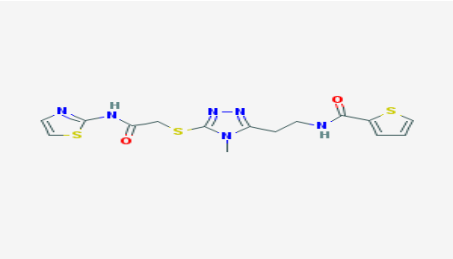
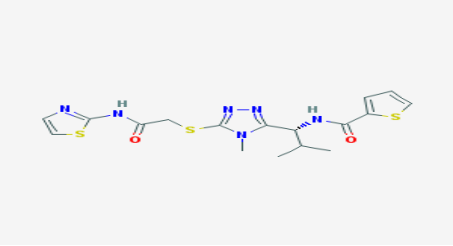
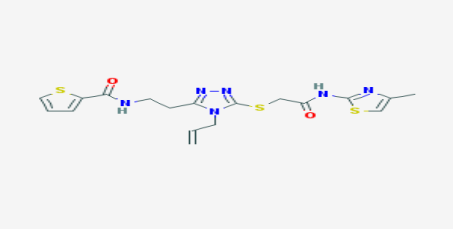
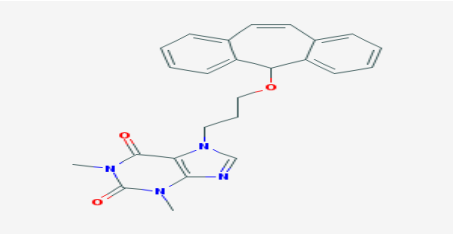
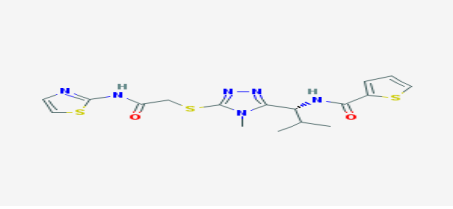
14	**CID 9542939**		**408.52**	**-6.2**	**HIS 232**
				-7.4	**ASP 455**
15	**CID 9542940**		**448.58**	**-5.6**	**HIS 232**
				-6.6	**ASP 455**
16	**CID 9542941**		**434.55**	**-5.6**	**HIS 232**
				-7.0	**ASP 455**
17	**CID 11838789**		**428.48**	**-7.1**	**HIS 232**
				-8.4	**ASP 455**
18	**CID 40478367**		**436.57**	**-6.5**	**HIS 232**
				-6.2	**ASP 455**

Table G: Toxicity of above Inhibitors against Lanosterol synthase

Sl No	PUBCHEM ID of Molecules	Toxicity Result	Organic Toxic Group Found
1	CID 1006318	Non Toxic	----
2	CID 2213568	Toxic	Aminothiazole Group
3	CID 2213595	Toxic	Aminothiazole Group
4	CID 2213690	Toxic	Aminothiazole Group
5	CID 2958449	Toxic	Aminothiazole Group
6	CID 2958467	Toxic	Aminothiazole Group
7	CID 3428244	Non Toxic	----
8	CID 4392376	Non Toxic	----
9	CID 4398863	Non Toxic	----
10	CID 4617056	Toxic	Aminothiazole Group
11	CID 6413427	Non Toxic	----
12	CID 9542937	Toxic	Aminothiazole Group
13	CID 9542938	Toxic	Aminothiazole Group
14	CID 9542939	Toxic	Aminothiazole Group
15	CID 9542940	Toxic	Aminothiazole Group
16	CID 9542941	Toxic	Aminothiazole Group
17	CID 11838789	Non Toxic	----
18	CID 40478367	Toxic	Aminothiazole Group

Table H: Drug Likeness (Log P and Molar Refractivity) of inhibitors of Lanosterol synthase

Sl No	PUBCHEM ID OF MOLECULES	Log P	Molar Refractivity
1	CID 1006318	3.48	118.050
2	CID 2213568	3.32	119.357
3	CID 2213595	3.34	114.598
4	CID 2213690	2.48	110.557
5	CID 2958449	3.32	119.357
6	CID 2958467	3.34	114.598
7	CID 3428244	4.97	129.935
8	CID 4392376	3.26	115.068
9	CID 4398863	3.54	122.916
10	CID 4617056	3.27	131.206
11	CID 6413427	2.12	85.29
12	CID 9542937	2.61	115.148
13	CID 9542938	2.26	110.400
14	CID 9542939	2.12	105.808
15	CID 9542940	2.99	119.562
16	CID 9542941	2.86	114.917
17	CID 11838789	3.34	124.032
18	CID 40478367	3.34	114.598

Table I: Cross Docking (Using the natural ligands docked with our previous target HMGCoA reductase against Lanosterol synthase)

Sl No	Name of the natural Compound	Binding Energy (K.cal/mol)
1	Apigenin	-8.9
2	Naringenin	-8.7
3	Epigallo catechin Gallate	-7.5
4	β-carotene	-4.9
5	Ascorbic acid	-5.3
6	Eugenol	-6.5
7	Glutathione	-6.3
8	Hesperetin	-6.5
9	Lutein	-4.4

10	**α-carotene**	**-4.4**
11	**Pantothenic acid**	**-6.2**
12	**Pyridoxine**	**-5.7**
14	**Riboflavin**	**-5.8**
15	**Thiamine**	**-5.2**
16	**Tocoferol**	**-5.6**
17	**Uric acid**	**-6.3**

6.6 CROSS DOCKING RESULTS: (Showing **a. Apigenin and b. Naringenin** with best Binding energy results with Lanosterol synthase)

a. Apigenin

```
#################################################################
# If you used AutoDock Vina in your work, please cite:          #
#                                                               #
# O. Trott, A. J. Olson,                                        #
# AutoDock Vina: improving the speed and accuracy of docking    #
# with a new scoring function, efficient optimization and       #
# multithreading, Journal of Computational Chemistry 31 (2010)  #
# 455-461                                                       #
#                                                               #
# DOI 10.1002/jcc.21334                                         #
#                                                               #
# Please see http://vina.scripps.edu for more information.      #
#################################################################

Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -102457424
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -8.9      0.000      0.000
   2         -6.7     24.459     25.561
   3         -6.3     24.618     25.859
   4         -6.2     25.593     28.126
   5         -6.2     23.782     24.617
   6         -6.1     26.168     28.092
   7         -6.1     26.187     27.543
Writing output ... done.
```

b. Naringenin

```
#################################################################
# If you used AutoDock Vina in your work, please cite:          #
#                                                               #
# O. Trott, A. J. Olson,                                        #
# AutoDock Vina: improving the speed and accuracy of docking    #
# with a new scoring function, efficient optimization and       #
# multithreading, Journal of Computational Chemistry 31 (2010)  #
# 455-461                                                       #
#                                                               #
# DOI 10.1002/jcc.21334                                         #
#                                                               #
# Please see http://vina.scripps.edu for more information.      #
#################################################################

Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 461558768
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -8.7      0.000      0.000
   2         -8.4      2.041      2.694
   3         -6.7     23.540     25.437
   4         -6.2     26.180     28.227
   5         -6.1     26.954     29.066
   6         -6.0     23.611     25.665
   7         -6.0     23.256     24.031
   8         -5.9     23.436     25.046
   9         -5.9     26.228     28.402
Writing output ... done.
```

6.7 DOCKING RESULTS (Showing Quinuclidine inhibitors with PUBCHEM ID's **a. CID 4398863 b. CID 1138789 c. CID 1006318** with best binding energy results with Lanosterol synthase.)

a. CID 4398863

```
#################################################################
# If you used AutoDock Vina in your work, please cite:          #
#                                                               #
# O. Trott, A. J. Olson,                                        #
# AutoDock Vina: improving the speed and accuracy of docking    #
# with a new scoring function, efficient optimization and       #
# multithreading, Journal of Computational Chemistry 31 (2010)  #
# 455-461                                                       #
#                                                               #
# DOI 10.1002/jcc.21334                                         #
#                                                               #
# Please see http://vina.scripps.edu for more information.      #
#################################################################

Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 919162072
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -8.4      0.000      0.000
   2         -8.2      1.204      2.142
   3         -8.0      1.821      4.196
   4         -7.8      1.712      3.923
   5         -7.7      2.714      5.372
   6         -7.7     14.014     17.613
   7         -7.7      2.364      4.534
   8         -7.7      4.686      9.122
   9         -7.6     14.505     16.028
Writing output ... done.
```

b. CID 1138789

```
#################################################################
# If you used AutoDock Vina in your work, please cite:          #
#                                                               #
# O. Trott, A. J. Olson,                                        #
# AutoDock Vina: improving the speed and accuracy of docking    #
# with a new scoring function, efficient optimization and       #
# multithreading, Journal of Computational Chemistry 31 (2010)  #
# 455-461                                                       #
#                                                               #
# DOI 10.1002/jcc.21334                                         #
#                                                               #
# Please see http://vina.scripps.edu for more information.      #
#################################################################

Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -187943744
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -8.4      0.000      0.000
   2         -8.0      1.205      2.144
   3         -8.0      1.753      4.201
   4         -7.8      1.635      3.919
   5         -7.8      2.681      5.382
   6         -7.8      2.228      4.562
   7         -7.7     14.841     16.595
   8         -7.7      4.787      9.451
   9         -7.5     13.948     17.162
Writing output ... done.
```

c. CID 1006318

```
#################################################################
# If you used AutoDock Vina in your work, please cite:          #
#                                                               #
# O. Trott, A. J. Olson,                                        #
# AutoDock Vina: improving the speed and accuracy of docking    #
# with a new scoring function, efficient optimization and       #
# multithreading, Journal of Computational Chemistry 31 (2010)  #
# 455-461                                                       #
#                                                               #
# DOI 10.1002/jcc.21334                                         #
#                                                               #
# Please see http://vina.scripps.edu for more information.      #
#################################################################

Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 364708072
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -8.3      0.000      0.000
   2         -8.3     13.475     17.037
   3         -8.2      1.045      2.065
   4         -8.1     14.588     17.578
   5         -8.0      2.809      4.804
   6         -8.0      2.772      5.210
   7         -7.9      2.304      4.222
   8         -7.9     14.226     17.521
   9         -7.9      5.486      8.099
Writing output ... done.
```

6.8 DOCKING RESULTS (Showing natural molecules **a. Naringenin b. Hesperetin c. Apigenin** with best binding energy results with HMGCoA reductase)

a. Naringenin

```
###############################################################
# If you used AutoDock Vina in your work, please cite:          #
#                                                               #
# O. Trott, A. J. Olson,                                        #
# AutoDock Vina: improving the speed and accuracy of docking    #
# with a new scoring function, efficient optimization and       #
# multithreading, Journal of Computational Chemistry 31 (2010)  #
# 455-461                                                       #
#                                                               #
# DOI 10.1002/jcc.21334                                         #
#                                                               #
# Please see http://vina.scripps.edu for more information.      #
###############################################################

Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 1354094840
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -7.3      0.000      0.000
   2         -7.1      2.532      8.326
   3         -6.9     23.253     25.386
   4         -6.8     24.628     27.127
   5         -6.4     24.141     27.415
   6         -6.3      9.385     12.454
   7         -6.2     24.268     26.937
   8         -6.2     23.123     26.196
   9         -6.1     23.470     25.479
Writing output ... done.
```

b. Hesperetin

```
###############################################################
# If you used AutoDock Vina in your work, please cite:          #
#                                                               #
# O. Trott, A. J. Olson,                                        #
# AutoDock Vina: improving the speed and accuracy of docking    #
# with a new scoring function, efficient optimization and       #
# multithreading, Journal of Computational Chemistry 31 (2010)  #
# 455-461                                                       #
#                                                               #
# DOI 10.1002/jcc.21334                                         #
#                                                               #
# Please see http://vina.scripps.edu for more information.      #
###############################################################

Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -2039174004
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -7.3      0.000      0.000
   2         -7.1     23.545     25.346
   3         -6.8     23.888     26.243
   4         -6.8      2.189      7.327
   5         -6.7     25.621     27.717
   6         -6.5     25.438     28.306
   7         -6.4      2.333      7.725
   8         -6.2      1.445      2.977
   9         -6.2     24.774     26.815
Writing output ... done.
```

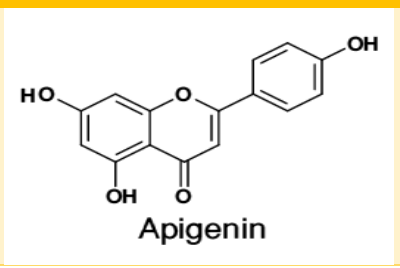
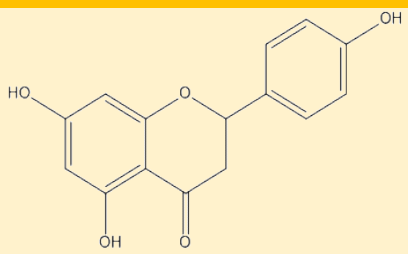
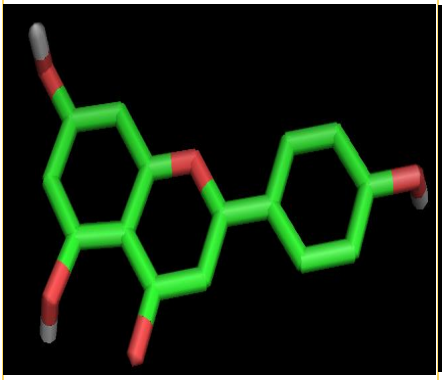
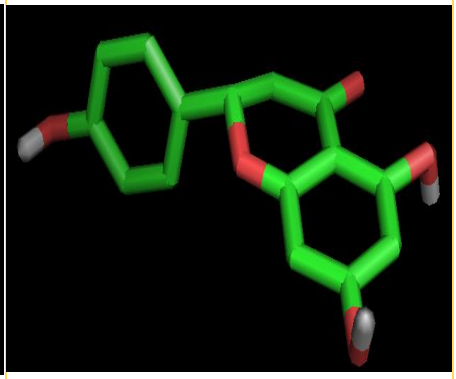
c. Apigenin

```
###############################################################
# If you used AutoDock Vina in your work, please cite:          #
#                                                               #
# O. Trott, A. J. Olson,                                        #
# AutoDock Vina: improving the speed and accuracy of docking    #
# with a new scoring function, efficient optimization and       #
# multithreading, Journal of Computational Chemistry 31 (2010)  #
# 455-461                                                       #
#                                                               #
# DOI 10.1002/jcc.21334                                         #
#                                                               #
# Please see http://vina.scripps.edu for more information.      #
###############################################################

Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -2020445088
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -7.2      0.000      0.000
   2         -7.1     23.811     25.906
   3         -6.8      1.977      7.312
   4         -6.7     23.167     26.258
   5         -6.5      2.386      8.185
   6         -6.5     24.419     27.429
   7         -6.5     25.716     27.788
   8         -6.4     26.637     29.505
   9         -6.3     24.719     26.692
Writing output ... done.
```

6.9 FINAL RESULTS (Two molecules Apigenin and Naringenin have shown best Binding Energy results on docking with both HMGCoA reductase and Lanosterol synthase. This shows they can inhibit pathway by acting against both targets**)**

	APIGENIN	NARINGENIN
CHEMICAL STRUCTURE	 Apigenin	
PYMOL VISUALIZED STRUCTURE		
Docking result with HMGCoA reductase	**-7.2 kcal/mol**	**-7.3 kcal/mol**
Docking result with Lanosterol synthase	**-8.9 kcal/mol**	**-8.7 kcal/mol**
Toxicity	**Non toxic**	**Non toxic**
Partition coefficient(LogP)	**2.71**	**2.84**
Molecular refractivity	**72.914**	**71.290**
Molecular formula	**$C_{15}H_{10}O_5$**	**$C_{15}H_{12}O_5$**
Molecular mass	**270.24 g/mol**	**272.2528 g/mol**

CHAPTER 7

DISCUSSION

7. DISCUSSION:

Hyperlipidemia is elevated level of cholesterol in the body. Major production of cholesterol takes place in the liver through Mevalonate pathway. Many enzymes HMGCoA synthase, HMGCoA reductase, Farnesyl PP synthase, Lanosterol synthase, Squalene synthase play a major role in the cholesterol biosynthesis pathway. So in our drug designing study, we have selected two important enzymes HMGCoA reductase and Lanosterol synthase as our targets. We found that there are already available drugs acting against HMGCoA reductase known as Statins. We obtained the active sites of our target using CASTP. After prediction of our active sites, we found the binding energies on docking HMGCoA reductase with Statins and results obtained was noted as POSITIVE CONTROL 1. There binding energies ranged from about -6.0 kcal/mol to -8 kcal/mol and this range is choosen as POSITIVE CONTROL 1. We then studied for some natural treatment and found some natural molecules which are known to lower cholesterol. We obtained the structures of those natural molecules. We docked them with HMGCoA reductase and compared the binding energies obtained with the POSITIVE CONTROL 1. We found that Apigenin, Naringenin, Hesperitin showed best results with binding energy values -7.2 kcal/mol, -7.3 kcal/mol, -7.3 kcal/mol. In the next stage, we targeted another enzyme Lanosterol synthase responsible for the same pathway in later stages. We searched for known inhibitors available against Lanosterol Synthase and found a class of Quinuclidine inhibitors acting against Lanosterol Synthase. We obtained the structures of those inhibitors, predicted the active sites of Lanosterol synthase and docked them with Lanosterol Synthase. We found the binding energies obtained were chosen as POSITIVE CONTROL 2 in this case. The binding energy values obtained are ranging from -8.4 kcal/mol to -6 kcal/mol. This is the noted POSITIVE CONTROL 2. Quinuclidine inhibitors CID 1006318, CID 4398863, CID 1138789 showed best binding energy results with Lanosterol Synthase. Their values are found to be -8.3 kcal/mol, -8.4 kcal/mol, -8.4 kcal/mol respectively. Now we have cross docked the previous natural ligands used against HMGCoA reductase with Lanosterol Synthase. We compared them with the POSlTIVE CONTROL 2 and noted the best results. We found that Apigenin and Naringenin showed highest Binding energy values with Lanosterol synthase. Their value are found to be -8.9 kcal/mol, -8.7 kcal/mol. We later predicted the toxicity and druglikeness of all inhibitors we used for docking. Apigenin and Naringenin are found to be non toxic and had Log P value 2.71 and 2.64 respectively.

These results show that both Apigenin and Naringenin prevent the pathway by inhibiting at both places of the cholesterol biosynthesis pathway. Druglikeness properties show that both Apigenin and Naringenin can act like drugs against Hyperlipidemia .

CHAPTER 8

CONCLUSION

8. CONCLUSION

We have targeted two enzymes responsible for the cholesterol biosynthesis pathway in liver. They are HMGCoA reductase and Lanosterol synthase. We have searched for already available drugs in the market acting against HMGCoA reductase called Statins. In our docking study, we have predicted the active sites of HMGCoA reductase. We docked HMGCoA reductase with Statins and binding energy values were taken as positive control. We then studied for some natural treatment and found some natural molecules which are known to lower cholesterol. We obtained the structures of those natural molecules. We docked them with HMGCoA reductase and compared the binding energies obtained with the positive control. In the next stage, we targeted another enzyme Lanosterol synthase responsible for the same pathway in later stages. We searched for known inhibitors available against Lanosterol Synthase and found a class of Quinuclidine inhibitors acting against Lanosterol Synthase. We obtained the structures of those inhibitors and docked them with Lanosterol Synthase. The binding energies obtained were chosen as positive control in this case. Now we cross docked the previous natural ligands used against HMGCoA reductase over Lanosterol Synthase. We compared them with the positive control and noted the best results. We later predicted the toxicity and druglikeness of all inhibitors.

We found that natural molecules Apigenin and Naringenin show best binding energy results with Lanosterol Synthase. Quinuclidine inhibitors CID 1006318, CID 4398863, CID 1138789 showed best binding energy results with Lanosterol Synthase. Natural molecules Apigenin, Naringenin, Hesperetin showed best results with HMGCoA reductase.

We found two natural molecules Apigenin and Naringenin inhibiting the pathway at both stages

CHAPTER 9

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9. REFERENCES

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